# Obesityand female infertility

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Walter Kuchenbecker

Cover design and lay-out: Venus of Willendorf illustrated by Gabriele Kuchenbecker.
The Venus of Willendorf is an 11 cm high statuette of a female figure carved out of limestone about 23,000 BC; it was most likely used as a fertility symbol.



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# Obesity and female infertility

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*"Fatness and flabbiness are to blame.*
*The womb is unable to receive the semen*
*and they menstruate infrequently....."*

*Hippocrates*
*in an essay to Scynthians 4th century B.C.*

# Contents

# Abbreviations

| | |
|---|---|
| ART | assisted reproductive techniques |
| BMI | body mass index |
| CT | abdominal computerised tomography scan |
| DEXA | dual-energy X-ray absorptiometry |
| DOHaD | developmental origin of health and adult disease |
| ESHRE | European Society of Human Reproduction and Embryology |
| FFA | free fatty acid |
| GEE | generalised estimating equations |
| HMW | high molecular weight |
| HOMA-IR | homeostasis model assessment score for insulin resistance |
| IR | insulin resistance |
| IL-6 | interleukin-6 |
| IAF | intra-abdominal fat |
| LAGB | laparoscopic adjustable gastric banding |
| LRYGB | laparoscopic Roux-en-Y gastric bypass |
| LSG | laparoscopic sleeve gastrectomy |
| MRI | magnetic resonance imaging |
| NTD | neural tube defects |
| PCOS | polycystic ovary syndrome |
| SHBG | sex hormone-binding globulin |
| ssCT | single-sliced abdominal CT scan |
| RCT | randomised controlled trial |
| SAF | subcutaneous abdominal fat |
| SFT | skinfold thickness |
| TNFα | tumour necrosis factor α |
| UMCG | University Medical Center Groningen |
| US | abdominal ultrasound |
| Wc | waist circumference |
| WHO | World Health Organisation |

# Chapter 1

# Introduction

In the past 30 years, the prevalence of overweight (BMI ≥ 25 kg/m$^2$) and obesity (BMI ≥30 kg/m$^2$) (see Table 1 for weight categories in adults) is on the rise worldwide, and due to its impact on health and disease it constitutes a global epidemic according to the World Health Organisation (WHO) (WHO, 2000).

The prevalence of obesity in women older than 15 years varies between regions (Figure 1A) and also between developed countries (Figure 1B) (WHO, 2012). In 2010, of all women in The Netherlands between the ages of 30 to 40 years, 32.2% were suffering from overweight and 9% from obesity (CBS, 2010). Considering the even stronger increase in the prevalence of childhood obesity in The Netherlands, a significant increase in obesity-related infertility can be anticipated in the future (Schokker *et al*., 2007). Gynaecologists will encounter the consequences associated with obesity more often due to a higher frequency of these women presenting with infertility or complications occurring during pregnancy.

**Obesity and female fertility**

Polycystic Ovary Syndrome (PCOS) is the most common cause of anovulation. It affects 4–7% of women of reproductive age and 61% of these women are overweight or obese (Pasquali *et al*., 2006). The association between obesity and ovulatory dysfunction has been well documented in the literature (Table 2).

Obesity in women with PCOS aggravates the underlying insulin resistance (IR) and the resulting hyperinsulinaemia and hyperandrogenaemia contribute to anovulation, although other obesity-related mechanisms contributing to anovulation cannot be excluded (Pasquali *et al*., 2006). Leptin, a peptide secreted by adipose tissue, may exert a direct inhibitory effect on ovarian function and interfere with the development of the dominant follicle and oocyte maturation (Duggal *et al*., 2000). The increased time to pregnancy (reduced fertility) observed in women with obesity is not solely attributable to anovulation, because obesity also decreases the chances of spontaneous conception in ovulatory women (Table 2) (Jensen *et al*., 1999, van der Steeg *et al*., 2008).

**Table 1.** WHO classification of weight categories in adults (adapted from WHO, 2010)

| BMI | Classification |
|---|---|
| BMI <18.5 | underweight |
| BMI 18.5–24.9 | normal weight |
| BMI 25–29.9 | overweight |
| BMI 30–34.9 | class I obesity |
| BMI 35–39.9 | class II obesity |
| BMI ≥40 | class III obesity (morbid obesity) |

Abbreviations: Body mass index (BMI) as kg/m$^2$.

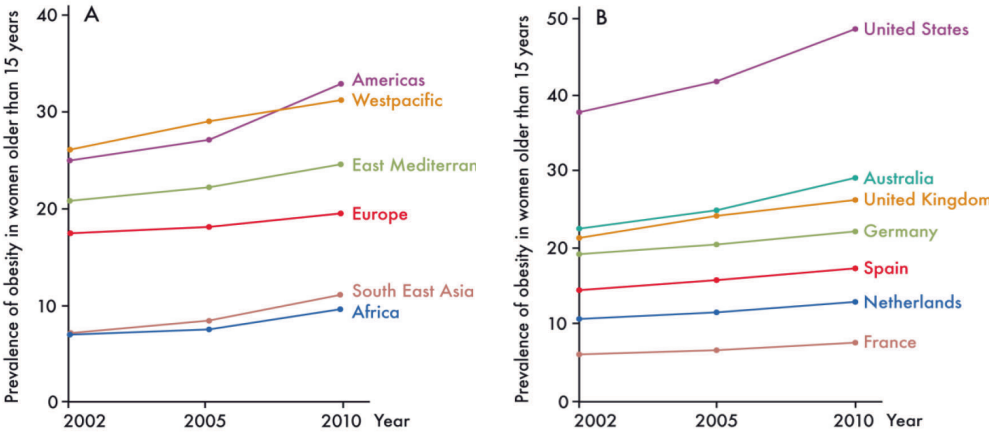


**Figure 1.** Prevalence of obesity in women older than 15 years of age in different regions (A) and in different developed countries (B). Derived from the global WHO database (WHO, 2012).

Obesity can also have an unfavourable effect on the outcome of fertility treatment. Obesity is considered an important predictor of unsuccessful ovulation induction in women with anovulatory infertility (Mulders *et al*., 2003). The effect of obesity on the pregnancy rate and live birth rate after ART (Assisted Reproductive Techniques) has been summarised in Tabel 2. There is an increased requirement for using gonadotrophins (gonadotrophin-based fertility-enhancing drugs), a higher miscarriage rate and a significant decrease in live birth rate with increasing BMI. The effect of obesity on live birth rate after ART was less pronounced in older women (>35 years) (Sneed *et al*., 2008; Luke *et al*., 2011), suggesting that the effect of age on the oocyte quality has a more detrimental effect on the live birth rate in older women than BMI. Not all studies agree whether the decrease in pregnancy rate and live birth rate after ART in women with obesity is determined by embryo quality , (Metwally *et al*., 2007; Bellver *et al*., 2010). Some studies in donoroocyte programs did not find a negative effect of obesity in the acceptor on pregnancy rates *(Wattanakumtornkul et al*., 2003; Styne-Gross *et al*., 2005), but others revealed a significant decrease in ongoing pregnancy rates with increasing BMI of the acceptor, in spite of no difference in embryo quality (Bellver *et al*., 2007). The high insulin levels as well as the changed serum adipokine levels (secretory products of adipose tissue) in women with obesity could influence the receptivity of the endometrium. IR has been shown to be an independent risk factor for spontaneous abortion (Tian *et al*., 2007). Hyperinsulinaemia leads to reduced glycodelin and insulin growth factor binding protein, both associated with the disruption of the adhaesion at the embryo-maternal interface (Carrington *et al*., 2005).

**Table 2.** Overview of the literature on the effect of obesity on female reproduction

| Outcome | Study type | n | Magnitude of effect | Reference |
|---|---|---|---|---|
| Anovulation | Case control | 2527 | BMI ≥30 RR 2.7 (95% CI: 1.8–3.6) | Rich-Edwards et al., 1994 |
| | Case control | 597 | BMI ≥27 RR 3.1 (95% CI: 2.2–4.4) | Grodstein et al., 1994 |
| Spontaneous ongoing pregnancy rate | Prospective cohort | 3029 ovulatory women | For each BMI unit above 29, OR 0.96 (95% CI: 0.91–0.99) | van der Steeg et al., 2008 |
| ART - clinical pregnancy rate | Systematic review | 2883 | BMI ≥25 OR 0.71 (95% CI: 0.62–0.81) | Maheshwari et al., 2007 |
| ART - decrease in live birth rate | Retrospective cohort | 45,163 ART embryo transfer cycles | BMI ≥30 adjusted OR 1.36–1.48 (95% CI: 1.10–1.99) in all women<br>BMI ≥30 adjusted OR 1.36–1.48 (95% CI: 1.23–2.48) in women ≤35 yrs<br>BMI ≥30 adjusted OR 1.26–1.34 (95% CI: 0.86–2.04) in women >35 yrs | Luke et al., 2011 |
| Miscarriage | Systematic review | 28,538 spontaneous pregnancies | BMI ≥28 OR 1.31 (95% CI: 1.18–1.46) | Boots & Stephenson, 2011 |
| | Retrospective cohort | 413 ART pregnancies | BMI ≥25 adjusted OR 2.7 (95% CI: 1.5–4.9) | Rittenberg et al., 2011 |
| | Retrospective cohort | 844 spontaneous pregnancies in recurrent pregnancy loss | BMI ≥30 OR 1.71 (95% CI: 1.05–2.8) for miscarriage in subsequent pregnancy | Metwally et al., 2010 |
| Congenital anomalies | Systematic review | 2093 - 9349 | BMI ≥30 OR 1.87 (95% CI: 1.62–2.15) for neural tube defects, OR 2.24 (95% CI: 1.86-2.69) for spina bifida, OR 1.30 (95% CI: 1.12–1.51) for cardiovascular anomalies | Stothard et al., 2009 |
| Pregnancy complications | Prospective cohort | 16,102 | BMI 30–34.9 OR 2.6 (95% CI: 2.1–3.4) for gestational diabetes, OR 1.6 (95% CI: 1.1–2.25) for pre-eclampsia, OR 2.0 (95% CI: 1.4–3.0) for macrosomia<br>BMI ≥35 OR 4.0 (95% CI: 3.1–5.2) for gestational diabetes, OR 3.3 (95% CI: 2.4–4.5) for pre-eclampsia | Weiss et al., 2004 |
| | Prospective cohort | 3480 | BMI ≥40 OR 4.82 (95% CI: 4.04–5.74) for pre-eclampsia, OR 2.79 (95% CI: 1.94–4.02) for intra-uterine death, OR 2.69 (95% CI: 2.49–2.90) for caesarean delivery, OR 1.34 (95% CI: 1.16–1.56) for instrumental delivery, OR 3.14 (95% CI: 1.86–5.31) for shoulder dystocia, OR 3.41 (95% CI: 2.07–5.63) for early neonatal death | Cedergren, 2004 |
| | Retrospective cohort | 287,213 | BMI ≥30 OR 1.7 (95% CI: 1.64–1.76) for induction of labour, OR 1.83 (95% CI: 1.74–1.93) for emergency caesarean section, OR 1.39 (95% CI: 1.32–1.46) for postpartum haemorrhage | Sebire et al., 2001 |

Abbreviations: Assisted reproduction (ART), Body mass index (BMI) as kg/m$^2$.

A limited amount of studies could not show an increase in complication rates following ART (such as haemorrhage, infection and injury to pelvic structures) in women who are overweight or obese compared to women of normal weight (Koning *et al*., 2012). Considering the small sample size of above-mentioned studies, more studies are needed to confirm that complication rates following ART are not increased in women who are overweight or obese.

***Obesity and pregnancy***

Women with obesity are more likely to experience miscarriages after spontaneous conception as well as after ART, and women with obesity and recurrent miscarriages have a significantly higher chance of miscarriage in a subsequent pregnancy compared to women of normal weight (Metwally *et al*., 2010) (Table 2).

Maternal obesity has a detrimental effect on fetal development resulting in an increased risk of congenital anomalies, especially neural tube defects (NTD) and cardiac malformations (Table 2). Folic acid fortification to decrease the risk of NTD has lower benefit in obese vs. non-obese women (Werler *et al*., 1996; Ray *et al*., 2005). Prenatal diagnosis of congenital abnormalities is less accurate in women with obesity due to poor ultrasound visualisation (Hendler *et al*., 2004; Hendler *et al*., 2005). The obese pregnant woman is exposed to increased risks during pregnancy, delivery and in the postpartum period (Table 2). The obstetric anaesthesia-related problems of obesity, like failed epidural or spinal anaesthesia and failed intubation, often performed under emergency circumstances, poses serious risks to the mother and the fetus (Hall and Neubert, 2005). The Confidential Enquiry into Maternal and Child Health in the United Kingdom indicates that 35% of the maternal deaths were obese compared to 23% of women of the general population, and that more than 25% of maternal deaths had a BMI >35 kg/m$^2$ (Lewis, 2007).

An additional concern of maternal obesity is that it may predispose to long-term disease in the offspring due to the developmental origin of health and adult disease (DOHaD) (Wadhwa *et al*., 2009), also known as the 'Barker's hypothesis' (Barker *et al*., 1989; Barker *et al*., 1993). According to the DOHaD hypothesis, the intra-uterine environment may induce fetal adaptive responses leading to altered physiologic and homeostatic set points mediated by epigenetic changes and contributing to the risk of adult onset of disease (Symonds *et al*., 2009; Wadhwa *et al*., 2009; Hanson *et al*., 2011). Research in animal models reveal that maternal hyperglycaemia contributes to adult onset IR and liver-fat accumulation as well as compromised long-term bone health in the off-spring (Zhao and Weiler, 2010; Song *et al*., 2012,).

In conclusion, maternal obesity contributes to adverse perinatal outcomes as well as to an increase in maternal morbidity and mortality. The female obesity-related reproductive consequences should therefore be a priority in clinical practice and in research programmes.

***Body-fat distribution and abdominal fat compartments***

Obesity is a heterogeneous condition and 20% of obese individuals have a metabolically healthy obese phenotype, which does not have the burden of obesity-associated cardio-metabolic risk factors (Hayes *et al*., 2010). It has been hypothesized that the detrimental effects of obesity on short- and long-term health risks are especially determined by differences in body-fat distribution. Accumulation of fat predominantly on the hips and thighs, called peripheral or gynaecoid obesity, poses little metabolic risks (Haslam and James, 2005; Despres *et al*., 2008). Accumulation of fat around the abdomen, called abdominal obesity (also called android or upper body obesity) is associated with metabolic complications such as cardiovascular disease and type 2 diabetes mellitus (Haslam and James, 2005; Hayes *et al*., 2010). Moreover, abdominal obesity also plays a role in female infertility and pregnancy complications (Pasquali *et al*., 2003; Carmina *et al*., 2007; Nelson and Fleming, 2007). Abdominal obesity contributes to reproductive dysfunction mainly by contributing to increased IR. High insulin levels interfere with intra-ovarian steroidogenesis leading to hyperandrogenaemia and arrest of follicle growth (Franks *et al*., 2008). Hyperandrogenaemia may alter adipose tissue mass in a depot-specific manner. The mechanism of this effect is not clear yet, but may be based on site-specific modulation of preadipocyte proliferation and/or differentiation as well as lipid synthesis and/or lipolysis in mature adipocytes (Blouin *et al*., 2008). Chronic hyperandrogenaemia may favour abdominal fat accumulation in women, triggering a vicious circle of abdominal obesity, hyperinsulinaemia and hyperandrogenaemia (Pasquali *et al*., 2006).

Abdominal fat consists of an intra-abdominal fat (IAF) and a subcutaneous fat (SAF) compartment. IAF consists mostly of mesenteric and omental fat. The products of IAF enter the portal venous system and arrive firstly at the liver, whereas the venous drainage of SAF is into the peripheral circulation. IAF and SAF also show differences in metabolic activity. Compared to SAF, IAF shows higher lipolytical activity (Weiss, 2007). Therefore, in women with an enlarged IAF mass, the elevated portal flux of free fatty acids, glycerol and other substances have a detrimental effect on hepatic metabolism leading to increased gluconeogenesis, decreased insulin clearance and decreased production of sex hormone binding globulin (Weiss, 2007). SAF on the other hand acts as a metabolic sink by buffering elevated postprandial fatty acid and lipid fluxes and by storage of excess triglycerides (Weiss, 2007; Koska *et al*., 2008). It has been shown that an excess of IAF contributes to an increased risk of developing cardio-metabolic disorders (Hayes *et al*., 2010). In premenopausal women with abdominal obesity, IAF is associated with IR even after correcting for SAF (Ross *et al*., 2002). Studies on the role of IAF on female reproduction are limited. IAF is considered a marker for IR and cardiovascular risk in obese women with PCOS (Lord *et al*., 2006). Increased IAF during early pregnancy is associated with an increase in IR, increased diastolic blood pressure and unfavourable lipid profile,

and it has also been shown to predict glucose intolerance in later pregnancy (Bartha *et al*., 2007; Martin *et al*., 2009).
Only limited studies have been performed to assess whether accumulation of IAF during the pre-conception period and in pregnant women has an adverse influence on reproductive and fetal outcome. No previous studies have been performed on the effect of lifestyle intervention on IAF accumulation and the outcome in infertile and pregnant women with obesity.

***Measurement of body-fat distribution and of IAF and SAF***
Methods available to measure obesity and body-fat distribution differ in accuracy, feasibility, safety and costs. BMI as a crude measure of obesity gives no information on body-fat distribution (National Heart, Lung and Blood Institute/National Institutes of Diabetes and Digestive and Kidney diseases, 1998; Prentice and Jebb, 2001). In epidemiological studies, waist circumference measurement is used to identify individuals with abdominal obesity (Janssen *et al*., 2002; Zhang *et al*., 2008), but it cannot distinguish IAF from SAF (Despres, 2006; Weiss, 2007; Jensen, 2008). Dual-energy X-ray absorptiometry (DEXA) scan can quantify body-fat distribution accurately, but it cannot distinguish between IAF and SAF. Abdominal Computerised Tomography scan (CT) or Magnetic Resonance Imaging (MRI) are the gold standards for the measurement of IAF and SAF (Figure 2), but pose limitations due to radiation exposure, costs and access to the equipment (Seidell *et al*., 1990). Several studies in different populations have confirmed that abdominal ultrasound (US) is a reliable and valid tool for the measurement of IAF and SAF when compared to CT and MRI (Armellini *et al*., 1993; Stolk *et al*., 2001; Gradmark *et al*., 2010). These studies were performed in populations with increased metabolic risk, but not in women of reproductive age with overweight or obesity. Furthermore, only one study tried to validate the measurement of the changes of IAF and SAF between US and CT in study subjects undergoing weight loss (Armellini *et al*., 1991). Before the implementation of US to measure IAF and SAF in women of reproductive age, validation studies are needed because body composition and abdominal fat distribution change with age and vary between different populations.

***Adipose tissue as an endocrine organ***
Adipose tissue was traditionally thought to be a passive reservoir used for energy storage, but it is now known to be a complex and highly active endocrine organ (Kershaw and Flier, 2004). Besides adipocytes and pre-adipocytes, adipose tissue consists of connective tissue matrix, nerve tissue, stromovascular and immune cells. Adipose tissue contains enzymes involved in the metabolism of steroid hormones, and it produces various proteins and peptides, collectively called adipokines (Kershaw and Flier, 2004; Ahima, 2006; Bohler *et*

*al*., 2010). Adipokine quantities may vary by the metabolic activity of the adipose tissue, per fat compartment (IAF versus SAF) and per stage of cell development (preadipocytes versus adipocytes). Adipokines, like leptin, adiponectin, interleukin-6 and tumor necrosis factor α, act in an autocrine and paracrine fashion on adipose tissue itself, and in an endocrine manner on distant tissues and organs. They have been suggested to influence reproductive function due to their effect on hypothalamic function and direct effects on the ovary and endometrium (Kershaw and Flier, 2004; Bohler *et al*., 2010). Serum levels of adipokines could therefore be used to assess the metabolic activity and effect of different adipose tissue compartments on female reproduction. Further studies are needed to assess whether serum adipokine levels correlate with the volume of IAF and SAF, and whether the serum adipokine levels can be used to measure the metabolic activity and effect of IAF and SAF on reproductive outcome.

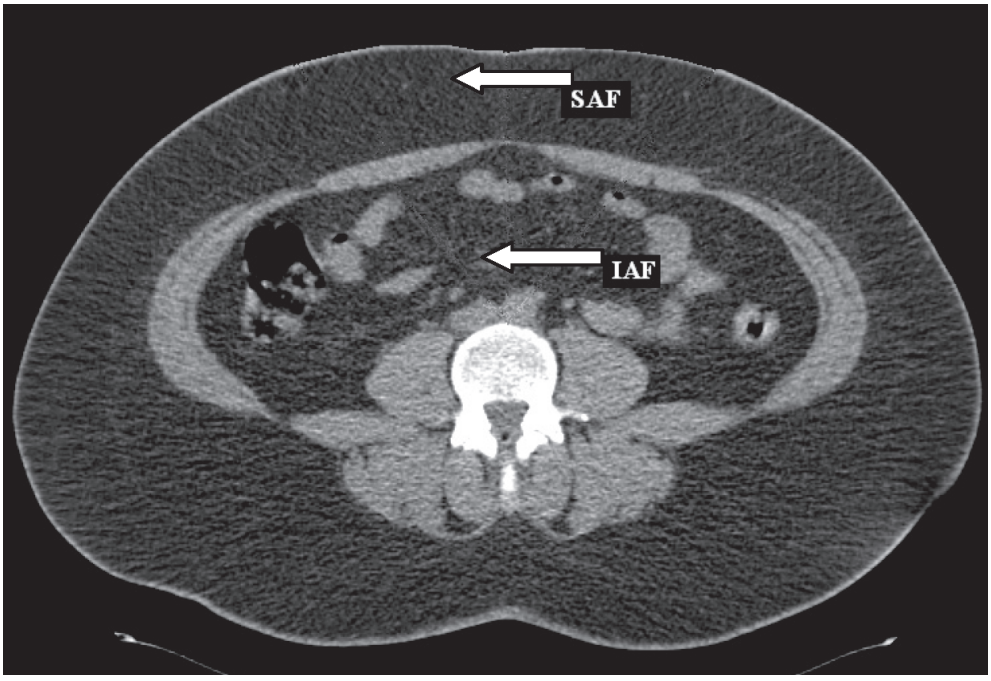


**Figure 2.** CT scan showing intra-abdominal fat (IAF) and subcutaneous abdominal fat (SAF). With courtesy of Mr. Wim Tukker, Department of Radiology, University Medical Center Groningen.

***Weight loss for resumption of ovulation***

The management of obesity should include measures to help patients lose weight and maintain appropriate body weight, thus lowering their cardiovascular and metabolic risks and putatively enhance their chances of conception. Weight loss programmes in women with anovulation have shown that 5–10% loss of initial body weight leads to resumption of ovulation in about 60% of women by reducing circulating insulin levels and reducing hyperandrogenaemia (Kiddy *et al*., 1992; Guzick *et al*., 1994; Clark *et al*., 1995). In women with PCOS, 5% loss of abdominal fat is associated with resumption of ovulation (Huber-Buchholz *et al*., 1999). During modest weight loss in women with obesity, loss of IAF had a greater beneficial effect on IR than loss of SAF (Park and Lee, 2005). It is important to investigate whether it is the loss of IAF and not SAF that contributes to resumption of ovulation in anovulatory women with obesity. The results of such studies can facilitate future lifestyle intervention programmes targeting the loss of IAF.

***Medication as an adjunct to lifestyle intervention***

Lifestyle intervention programmes aiming at achieving and maintaining weight loss are usually modestly successful due to poor adherence to dietary and exercise measures and high drop-out rates (Finley *et al*., 2007). Drop-outs tend to have less weight loss (Finley *et al*., 2007), and therefore drop-out is an important limiting factor in the success of lifestyle intervention programmes (Messier *et al*., 2010). Weight loss medication as an adjunct to lifestyle invention has been shown to achieve more weight loss than lifestyle intervention alone, and up to 10% loss of initial body weight has been reported over a 1 year period (Vetter *et al*., 2010). Orlistat, a Food and Drug Administration approved weight loss drug, cannot be used in women who anticipate conception because of lack of safety data during its use in early pregnancy. Sibutramine, a second weight loss drug has been withdrawn from the American and European markets out of safety concerns. Insulin-sensitizing drugs are not considered weight loss drugs, even though some studies indicate that metformin might contribute to weight loss (Knowler *et al*., 2002). In anovulatory women with PCOS, metformin was studied extensively and may contribute to resumption of ovulation in a subset of patients (Tang *et al*., 2012). If insulin-sensitizing drugs can be shown to contribute to weight loss in women of reproductive age, they should be considered to be used as an adjunct to lifestyle intervention.

***Aims and outline of the thesis***

The studies in this thesis are undertaken to assess various aspects of obesity and female infertility with the main focus on the role and influence of body-fat distribution and especially IAF and SAF on female infertility.

There is a need to assess the costs and effects of overweight and obesity on female infertility and pregnancy outcome, in order to aid further research on the cost-effectiveness of lifestyle intervention and to formulate evidence based guidelines for the treatment of these women. In **Chapter 2,** the consequences of overweight and obesity with respect to fecundity, costs of fertility treatment and pregnancy outcome was studied in women with infertility.

Studies on the role IAF and SAF in female infertility are very limited. In **Chapter 3,** we measured body-fat distribution and compared the contribution of IAF and SAF to anovulation in obese women with infertility.

CT is the gold standard for the measurement of IAF and SAF, but US could be an ideal alternative due to the absence of radiation exposure, low cost and its general availability in most fertility and antenatal clinics. In **Chapter 4,** we investigated the correlation between the measurements of IAF and SAF by US and CT scan in women with obesity and infertility.

Studies are needed to assess whether serum adipokine levels can reflect the differences in body-fat distribution and the differences in the volume and metabolic activity of IAF and SAF. In **Chapter 5,** we assessed whether PCOS status is a determining factor in the correlation between body-fat distribution parameters and serum adipokine levels in women with obesity and infertility.

Loss of abdominal fat is required for the resumption of ovulation in obese women with PCOS. The question remains, whether differential loss of IAF and SAF contributes to resumption of ovulation. In **Chapter 6,** we compared the changes in body-fat distribution and specifically IAF and SAF in a group of anovulatory women with PCOS and obesity who resumed ovulating to those who remained anovulatory during a lifestyle programme.

Obese individuals have great difficulty in losing weight and insulin-sensitizing agents may help to achieve more weight loss. In **Chapter 7,** we performed a systematic review in women of reproductive age who are overweight or obese to assess whether treatment with insulin-sensitizing drugs contributes to weight loss, compared to diet or a lifestyle modification programme.

In **Chapter 8,** the main findings of this thesis are summarised and recommendations for clinical practice and future research are provided.

***Patients and methods***

The data presented in Chapters 3 to 6 are based on a prospective cohort of 60 women with obesity and infertility who participated in a lifestyle programme at the UMCG between 2005 and 2009.

All participants received individualised dietary advice, and an individualised exercise programme was tailored to the ability and personal and social circumstances of each

participant. Individual guidance by a nurse practitioner consisted of visits to the outpatient clinic every 2 weeks, during which body weight was measured and compliance was assessed. Motivational counselling techniques were used to address problems of and resistance to lifestyle changes and advice on behavior modification was given. Body-fat distribution was assessed by anthropometrics, DEXA and CT at intake, after 3 months and after 6 months. At these time points, blood samples were also taken. Table 3 gives an overview of the patients and methods used in this thesis.

**Table 3.** Overview of 60 women with obesity and infertility participating in a lifestyle programme at the University Medical Center Groningen, included in the present analysis

| Chapter | n | Patients | Methods |
|---|---|---|---|
| Three | 57 | Women with infertility; three women excluded because of early drop-out, pregnancy and CT measurement difficulty. (BMI 37.4±5.4) | Baseline comparison between ovulatory and anovulatory women to determine the contribution of variables to anovulation |
| Four | 53 | Women for whom US and CT measurements were available at baseline, month 3 and month 6. (BMI 37.0±4.9) | Correlation between the measurements of IAF and SAF and the changes measured by US and CT |
| Five | 47 | 32 women with anovulatory PCOS and 15 ovulatory non-PCOS controls. (BMI 36.8±4.9) | Correlation between body-fat distribution parameters and serum adipokine levels |
| Six | 32 | Anovulatory women with PCOS. (BMI 37.5±5.0) | Changes in anthropometrics and IAF and SAF over time comparing women who resumed ovulating to those who remained anovulatory during the lifestyle programme |

Abbreviations: Abdominal ultrasound (US); Body mass index (BMI) as $kg/m^2$; Abdominal computerised tomography (CT); Intra-abdominal fat (IAF); Polycystic ovary syndrome (PCOS); Subcutaneous abdominal fat (SAF).

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Chapter 2

# Economic consequences of overweight and obesity in infertility: A framework for evaluating the costs and outcomes of fertility care

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## ABSTRACT

***Background***: Overweight and obesity are an epidemic in Western society, and have a strong impact on fertility. We studied the consequences of overweight and obesity with respect to fecundity, costs of fertility treatment and pregnancy outcome in subfertile women.

***Methods***: We searched the literature for systematic reviews and large studies reporting on the effect of weight on both fecundity and pregnancy outcome in subfertile women. We collected data on costs of treatment with ovulation induction, intra-uterine insemination and in vitro fertilisation, as well as costs of pregnancy complications. We calculated, for ovulatory and anovulatory women separately, the number of expected pregnancies, complications and costs in a hypothetical cohort of 1000 normal weight, overweight and obese women each.

***Results***: In our hypothetical cohort of 1000 women, compared to women with normal weight live birth was decreased by 14 and 15% (from 806 live births to 692 and 687 live births) in overweight and obese anovulatory women respectively, for ovulatory women it was decreased by 22 and 24% (from 698 live births to 546 and 531 live births), respectively. These outcomes were associated with an increase in the number of complications and associated costs leading to cost per live birth in anovulatory overweight and obese women were 54 and 100% higher than their normal-weight counterparts, for ovulatory women they were 44 and 70% higher, respectively.

***Interpretation***: Overweight and obese subfertile women have a reduced probability of successful fertility treatment and their pregnancies are associated with more complications and higher costs.


## INTRODUCTION

The prevalence of overweight and obesity varies in populations and is estimated to range from 5% in some developing countries to >30% in developed countries (James *et al*., 2004). The World Health Organisation defines overweight as a body mass index (BMI) ≥25 kg/m$^2$, and obesity as a BMI ≥30 kg/m$^2$ (WHO, 2000). Considering the trends in childhood obesity, a significant increase in obesity-related subfertility can be anticipated in the future (Schokker *et al*., 2007). Nowadays, the rate of obesity in women of child-bearing age is 12% in Western Europe and 25% in North America (Butler *et al*., 2004; Linné *et al*., 2004; Watson *et al*., 2005; Haslam and James, 2005).

The strongest obesity-related effect on fertility is anovulation. Polycystic ovarian syndrome (PCOS), the most noted cause of anovulation, is furthermore exacerbated by increased

insulin resistance and hyperinsulinaemia associated with overweight and obesity (Pasquali *et al*., 2007). In 65% of patients with PCOS, obesity therefore contributes to anovulation (Pasquali *et al*., 2003). On the other hand, even obese women with an ovulatory cycle have a lower chance of spontaneous conception (Jensen *et al*., 1999; Van der Steeg *et al*., 2007).
In cases of chronic anovulation, ovulation induction (OI) with clomiphene citrate in overweight and obese women results in lower ovulation rates (Imani *et al*., 1998) and lower cumulative live birth rates for women with a BMI >30 kg/m² (Legro *et al*., 2007). McClure *et al*. (1992) showed that in overweight women ovulation rates are lower due to higher cancellation rates, but if OI is successful no difference is found in pregnancy rates in different weight categories. Mulders *et al*. (2003) also found obesity to be associated with higher cancellation rates and substantially higher miscarriage rates leading to a lower live birth rate per started cycle. This decreased success rate is however not found in all studies (Balen *et al*., 2006).
The literature on the impact of body weight on the effectiveness of intra-uterine insemination (IUI) is just as for OI, inconsistent. Koloszar *et al*. (2002) showed a negative impact of increasing body weight on the success rates of IUI, but Wang *et al*. (2004) could not confirm this finding.
Furthermore, several retrospective studies have shown a negative impact of overweight and obesity in women on the outcome of in vitro fertilisation (IVF) (Lashen *et al*., 1999; Wang *et al*., 2000; Koloszar *et al*., 2002; Fedorcsak *et al*., 2004). The ongoing pregnancy rate and live birth rate is however consistently decreased especially due to an increased miscarriage rate in women with obesity (Wang *et al*., 2002; Lintsen *et al*., 2005; Maheshwari *et al*., 2007).
Apart from these obesity-related fertility problems, there is indisputable evidence that pregnancy in overweight and obese women is associated with an increased risk of complications, leading to higher maternal and neonatal morbidity and mortality and increased costs (Sebire *et al*., 2001; Cedergren *et al*., 2004; Linne *et al*., 2004). Pregnancy complications associated with obesity are hypertensive disorders, gestational diabetes, prolonged duration of labour, increased need of operative delivery, macrosomia, shoulder dystocia and increased blood loss (Garbaciak *et al*., 1985; Edwards *et al*., 1996; Weiss *et al*., 2004). Obesity is furthermore associated with an increased risk of adverse pregnancy outcomes such as unexplained still birth (Cnattingius *et al*., 1998; Linne *et al*., 2004; Kristensen *et al*., 2005) and neonatal admissions (Usha Kiran *et al*., 2005).
In view of the issues stated above, it is likely that overweight and obesity have a negative impact on the outcome as well as the costs of fertility treatment. The aim of this paper is to conceptualise the impact of overweight and obesity on fertility treatment and the resultant pregnancies, in terms of effectiveness, costs and cost-effectiveness.

## METHODS

We developed a framework within which the consequences of fertility treatment and outcomes of resultant pregnancy can be evaluated simultaneously for subfertile women in different body weight categories. We performed systematic reviews to obtain information on outcomes and costs to generate cost-effectiveness estimates for inclusion in decision analytic models. To do so, we searched the literature for evidence on the effect of obesity on spontaneous pregnancy chances, success of assisted reproduction technologies (ART), as well as pregnancy outcome.

We used the following electronic databases: PubMed, Embase, DARE and the Cochrane Library to initially search for systematic reviews on each of the subjects. In absence of reviews, we identified large, reliable studies.

To identify studies that reported on the association between obesity and spontaneous pregnancy chances we combined the key words ('obesity', 'overweight' or 'body mass index') and ('pregnancy' or 'fertility'). By adding the key words ('assisted reproduction technologies', 'intra-uterine insemination') and ('ovulation induction') we looked for studies reporting on the effect of obesity on these treatments. To identify studies reporting on the association between obesity and pregnancy outcome, we used the key words: ('obesity', 'overweight' or 'body mass index') and ('pregnancy outcome').

We included studies reporting on maternal morbidity as well as pregnancy outcome. The reported odds ratios (ORs) in the reviews were used, or if not available, calculated by using a 2 x 2 table cross-classifying BMI and one of the afore-mentioned outcomes. These ORs were used as input for calculating the additional impact of overweight and obesity on both fecundity as well as pregnancy.

The economic analysis was performed from a hospital perspective. Costs of fertility treatments were obtained from a series of Dutch studies that reported on the costs of OI, IUI and costs of IVF (Goverde *et al*., 2000; Eijkemans *et al*., 2005). Furthermore, we looked for studies reporting on costs of pregnancy in overweight women and costs of pregnancy complications in these women. To do so, we performed a search of several major journals in obstetrics and gynaecology for economic evaluations. We looked for studies that reported on the costs of each of the complications miscarriage, pre-eclampsia, gestational diabetes and caesarean delivery. We assumed no difference in multiple pregnancy rates between different weight categories (Esinler *et al*., 2008).

Next, we assessed the impact of overweight and obesity on the costs and effects of fertility treatments. To achieve this, we distinguished between the case of ovulatory women and the case of anovulatory women. For each of these situations, we considered women with normal weight, overweight, and obese women. According to the WHO normal weight is

defined as a BMI between 20 and 25 kg/m², overweight as a BMI between 25 and 30 kg/m² and obesity as a BMI over 30 kg/m². Because of differences in definitions of overweight and obesity in some studies we used in our review, we could not use the very strict BMI cut off points proposed by the WHO for our different weight groups.

We then constructed a theoretical model, simulating the situation where women were treated for their subfertility. For each of the six categories, i.e., anovulatory women with normal weight, anovulatory overweight women and anovulatory obese women and ovulatory women with normal weight, ovulatory overweight women and ovulatory obese women, we calculated the expected pregnancy rates, the expected number of fertility treatments and the expected number of pregnancy complications for a hypothetical group of 1000 women. We performed multiple sensitivity analyses on the following variables success rate of IVF (range 40% to 60%), success rate of OI (range 70% to 90%) and IUI (range 30% to 50%). With these figures we calculated and then plotted in two figures different success rates of ART against the costs per live birth in anovulatory and ovulatory women in different weight categories.

## RESULTS

***Literature identified***

The search for studies on the association between spontaneous pregnancy chances in overweight women revealed two reviews by Jensen and Gesink Law *et al*. as well as the study of Van der Steeg *et al*. (Jensen *et al*., 1999; Gesink Law *et al*., 2007; Van der Steeg *et al*., 2007). The results of these studies are shown in Table 1. Both reviews as well as the study of Van der Steeg *et al*. showed that overweight women take longer to conceive than normal-weight women. The reviews were retrospective studies in a cohort of women not seeking medical help for any subfertility, whereas Van der Steeg *et al*. studied women in fertility clinics. Based on these results, we assumed that among obese ovulatory women spontaneous pregnancy chances were 90% of those in normal weight or overweight women. Moreover, we assumed that spontaneous pregnancy chances prior to and in between ART cycles were 10% in all groups.

From the literature no unequivocal conclusion could be drawn about the influence of obesity on IUI. Whereas Wang *et al*. (2004) reported an increased probability of success of IUI in women with a BMI >30 kg/m$^2$, Koloszar *et al*. (2002) reported exactly the opposite, i.e. a decrease in success of IUI with increasing BMI. In view of these conflicting results on IUI, for purpose of this review we considered no effect of BMI on IUI.

**Table 1.** Spontaneous fecundity and pregnancy chances after reproductive treatment in different weight categories

| Author | Study period | Study population | Data collection | Outcome measure | Results |
|---|---|---|---|---|---|
| ***Spontaneous pregnancy*** | | | | | |
| Gesink Law | 1959–1965 | first planned pregnancy | retrospective | chance conceiving per cycle | OR 0.92 (95% CI 0.84–1.01) for BMI 25–29.9<br>OR 0.82 (95% CI 0.75–0.92) for BMI >30<br>OR 0.66 (95% CI 0.49–0.89) for primipari with BMI >30 |
| Jensen | 1972–1987 | first planned pregnancy | retrospective | chance conceiving per cycle | OR 0.77 (95% CI 0.70–0.84) for BMI >25 |
| van de Steeg | 2002–2004 | subfertile ovulatory | prospective | time to pregnancy <12 months | HR 0.95 (CI 0.91–0.99) per extra kg/$m^2$ from a BMI >29 |
| ***Intra-uterine insemination*** | | | | | |
| Wang | 1990–2000 | infertile couples undergoing IUI treatment | retrospective | chance conceiving per cycle | OR 1.5 (95% CI 1.1–1.9)* for BMI >30 |
| Koloszar | 1992–1998 | infertile ovulatory | prospective | pregnancy | OR 0.66 (95% CI 0.49–0.88)* for BMI 25–27<br>OR 0.42 (95% CI 0.25–0.73)* for BMI 28–36 |
| ***In vitro fertilisation*** | | | | | |
| Maheshwari | 1966–2006 | infertile women undergoing IVF | systematic review | pregnancy | OR 0.71 (CI 95% 0.62–0.81) for BMI >25<br>OR 0.68 (CI 95% 0.55–0.83) for BMI >30 |

IUI, intra-uterine insemination; IVF, in vitro fertilisation; OR, odds ratio; HR, hazard ratio; *, calculated OR.

Maheshwari *et al*. (2007) published a systematic review of the literature from 1960 until 2006 on the outcome of IVF for overweight and obese women. They reported an OR for pregnancy after IVF of 0.71 (95% CI 0.62–0.81) for women with a BMI 25 kg/m$^2$ compared to women with a BMI between 20 and 25 kg/m$^2$, and for women with a BMI >30 kg/m$^2$ even 0.68 (95% CI 0.55–0.83) (Table 1). We applied these odds ratios in our model. Maheshwari *et al*. (2007) also found that overweight women require more total units of gonadotrophins during hyperstimulation for IVF, but these additional costs were not considered in the present analysis.

We found one meta-analysis and three studies that reported on the impact of BMI on the effectiveness of OI in anovulatory women (Table 2) (Imani *et al*., 2002; Mulders *et al*., 2003; Al-Azemi *et al*., 2004; Balen *et al*., 2006). The study of Al-Azemi *et al*. (2004) showed a negative impact of obesity on live birth rate after OI with clomiphene citrate. The meta-analysis of Mulders *et al*. (2003) did not show a significant impact of BMI on the fecundity after OI with gonadotrophins. However, they found higher cancellation rates per cycle (OR 1.9) and higher miscarriage rates in the obese group (OR 3.1), thus leading to lower ongoing pregnancy rates per started cycle.

**Table 2.** Odds ratio's of pregnancy chances of overweight and obese anovulatory women following ovulation induction

| Author | Study period | Study population | Study design | Outcome measure | Results |
|---|---|---|---|---|---|
| Azemi | not reported | infertile women undergoing CC-OI | retrospective cohort study | live birth | OR 0.74* (0.39–1.4) for BMI 25–29<br>OR 0.15* (0.07–0.30) for BMI >30 |
| Mulders | 1986–2002 | infertile women undergoing gonadotrophin-OI | meta analysis | pregnancy | OR 1.22 (95% CI 0.77–1.93) obese vs lean women |
| | | | | cancellation rate | OR 1.86 (95% CI 1.13–3.06) |
| | | | | miscarriage rate | OR 3.05 (95% CI 1.45–6.44) |
| Balen | 2002–2003 | after 3 cycles CC failed OI → gonadotrophins | prospective cohort study | pregnancy | OR 1.3* (0.71–2.2) for BMI>25<br>OR 0.99* (0.48–2.0) for BMI >30 |
| Imani | 1993–1995 | infertile women undergoing CC-OI | prospective cohort study | ovulation | OR 0.92 (0.88–0.96) obese vs normal weight |
| | | | | live birth | OR 1.00 (0.97–1.04) |

CC, clomiphene citrate; OI, ovulation induction; BMI, body mass index; *, calculated OR.

Balen *et al*. (2006) studied anovulatory women with a BMI up to 35 kg/m$^2$ and also did not find a significant difference in pregnancy rates after OI with gonadotrophins in overweight and obese women compared to women of normal weight. Imani *et al*. (2002) found among anovulatory women a hazard ratio of 0.92 for obese versus lean women for ovulation after OI with clomiphene citrate, but they also did not find a difference in live birth chances between the weight groups. We therefore assumed in our analysis that there is no influence of BMI on pregnancy rates after OI in anovulatory women.
Table 3 shows the additional risk of obstetric complications due to overweight and obesity. We applied meta-analyses conducted by Chu *et al*. (2007a,b,c) and Cnossen *et al*. (2007). These studies report that there is an additional risk of stillbirth, caesarean delivery, pre-eclampsia and gestational diabetes with increasing BMI. Fedorcsak *et al*. (2004) studied the impact of overweight in women undergoing IVF treatment, and found an increased risk of miscarriage.

**Table 3.** Odds ratios of maternal and fetal complications in overweight and obese women

| Author | Study period | Study population | Study design | Outcome measure | Results |
|---|---|---|---|---|---|
| Fedorcsak | 1996–2002 | IVF/ICSI | retrospective cohort | Abortion <6 weeks | OR 2.0* (95% CI 1.1–3.7) for BMI >25 |
| Chu (b) | 1980–2005 | birth registries, clinical medical records etc. | meta analysis | stillbirth | OR 1.5 (95% CI 1.1–1.9) for overweight women<br>OR 2.1 (95% CI 1.6–2.7) for obese women |
| Cnossen | 1980–2006 | Cohort (prospective and retrospective) | meta analysis | Pre-eclampsia | LRs (95% CI) 1.7 (0.3 –11.9) for BMI > or = 25 and 0.73 (0.22–2.45) for BMI <25 (OR 2.3)<br>LRs 2.7 (1.0–7.3) for BMI > or = 35 and 0.86 (0.68–1.07) for BMI <35 (OR 3.7) |
| Chu (c) | 1980–2005 | Cohort (prospective and retrospective) | meta analysis | caesarean delivery | OR 1.5 (95% CI 1.3–1.6) for overweight women<br>OR 2.1 (95% CI 1.9–2.3) for obese women<br>OR 2.9 (95% CI 2.3–3.8) for severely obese women |
| Chu (a) | 1980–2006 | Cohort (prospective and retrospective) | meta analysis | gestational diabetes | OR 2.1 (95% CI 1.8– 4.2) for overweight women<br>OR 3.6 (95% CI 3.1–4.2) for obese women<br>OR 8.6 (95% CI 5.1–16) for severely obese women |

BMI, body mass index; OR, odds ratio; LR, likelihood ratio; *, calculated OR.

**Table 4.** Costs of pregnancy complications and ART per pregnancy

| Study (year) | Complication/treatment | Costs per pregnancy (€) |
|---|---|---|
| Chen (2001) | caesarean delivery | 3350* |
| Barton (2006) | hypertensive disorder | 8250* |
| Moss (2007) | gestational diabetes mellitus | 345* |
| Graziosi (2005) | miscarriage | 683 |
| Goverde (2000) | IVF | 1700* |
| Eijkemans (2005) | OI | 250 |
| Goverde (2000) | IUI | 450* |

*Calculated from US and AUS dollars and Dutch guilders.

Table 4 presents expected costs of fertility treatment and pregnancy complications. We used several studies on costs of pregnancy complications and calculated the costs presented in euro's using the current exchange rates (Chen *et al*., 2001; Graziosi *et al*., 2005; Barton *et al*., 2006; Moss *et al*., 2007). Furthermore, we used studies on costs in The Netherlands for OI, IUI and IVF treatment (Goverde *et al*., 2000; Eijkemans *et al*., 2005).

***Expected outcome and costs***

Table 5 shows the result when the model was applied on a hypothetical cohort of 1000 anovulatory women. Our model represents costs until birth, including the costs of delivery. In 1000 normal-weight anovulatory women, treatment with three cycles of OI and, if needed, followed by one or two cycles of IVF, would result in 900 pregnancies. Figure 1A shows that costs per live birth are higher for overweight and obese anovulatory women with different success rates of OI and IVF and these differences in costs are roughly constant over a large range of success rates.

Of these pregnancies 90 are expected to end in miscarriage and 810 women will have an ongoing pregnancy. The expected number of pregnancies complicated by pre-eclampsia, gestational diabetes and caesarean delivery, will be 81, 41 and 81, respectively, whereas 4 women will suffer stillbirth. Overall, 806 women are expected to deliver a child, for a total cost of € 2430 per woman, resulting in a cost of € 3016 per live birth.

From Table 5, it can also be seen that in overweight anovulatory women the effectiveness of treatment decreases, resulting in a decrease of the number of pregnancies and live births, an increase in costs and a relative increase of the number of complications. This results in a decrease in the number of live births of 114 (14%), and an expected increase in costs of almost € 800 (32%) per patient. For obese anovulatory women, these figures are slightly worse, as the number of live births decrease to 119 (15%), and the expected increase in cost of approximately € 1700 (71%) per patient as compared to normal-weight women.

**Table 5.** Hypothetical cohort of 1000 anovulatory women in different weight categories

| | Normal weight | Over-weight[a] | Obese[b] |
|---|---|---|---|
| Cohort | 1000 | 1000 | 1000 |
| Impact of overweight on OI (OR) | 1 | 1 | 1 |
| Pregnant after 3 cycles OI (baseline rate 80%) | 800 | 800 | 800 |
| Number of women undergoing IVF | 200 | 200 | 200 |
| Impact of weight on effectiveness IVF (OR) | 1 | 0.71 | 0.68 |
| Pregnant after 2 cycles IVF (baseline rate 50%) | 100 | 71 | 68 |
| Expected number of pregnancies | 900 | 871 | 868 |
| Impact of weight on miscarriage (OR) | 1 | 2 | 2 |
| Expected number of miscarriages (baseline rate 10%) | 90 | 174 | 174 |
| Number of women without ongoing pregnancy | 190 | 303 | 306 |
| Number of women with ongoing pregnancy | 810 | 697 | 694 |
| Impact of weight on pre-eclampsia (OR) | 1 | 2.3 | 3.7 |
| 10% pregnancies complicated by pre-eclampsia | 81 | 160 | 257 |
| Impact of weight on gestational diabetes (OR) | 1 | 2.1 | 3.6 |
| 5% pregnancies complicated by gestational diabetes | 41 | 73 | 125 |
| Impact of weight on caesarean deliveries (OR) | 1 | 1.5 | 2.1 |
| 10% pregnancies caesarean delivery | 81 | 105 | 146 |
| Impact of weight on stillbirth (OR) | 1 | 1.5 | 2.1 |
| 0.5% pregnancies stillbirth | 4 | 5 | 7 |
| Total women with live birth | 806 | 692 | 687 |
| Total costs complications (€) | 1000 | 1788 | 2724 |
| Total expected cost (*€1000) | 2430 | 3218 | 4154 |
| Cost per live birth (€) | 3016 | 4653 | 6045 |
| Cost per pregnancy (€) | 3001 | 4618 | 5982 |

[a] Applied BMI (kg/m²) threshold differed from study to study (range 25–27).
[b] Applied BMI (kg/m²) threshold differed from study to study (range 29–35).

Table 6 shows the results for a theoretical cohort of 1000 ovulatory women. In 1000 normal-weight ovulatory women, treatment consisted of three cycles of IUI and if this was unsuccessful one or two cycles of IVF, added with 10% spontaneous pregnancies that occur on waiting lists or in between cycles, would result in 780 pregnancies. Figure 1B shows that over a large range of different success rates of IUI and IVF the costs per live birth are higher for overweight and obese ovulatory women.

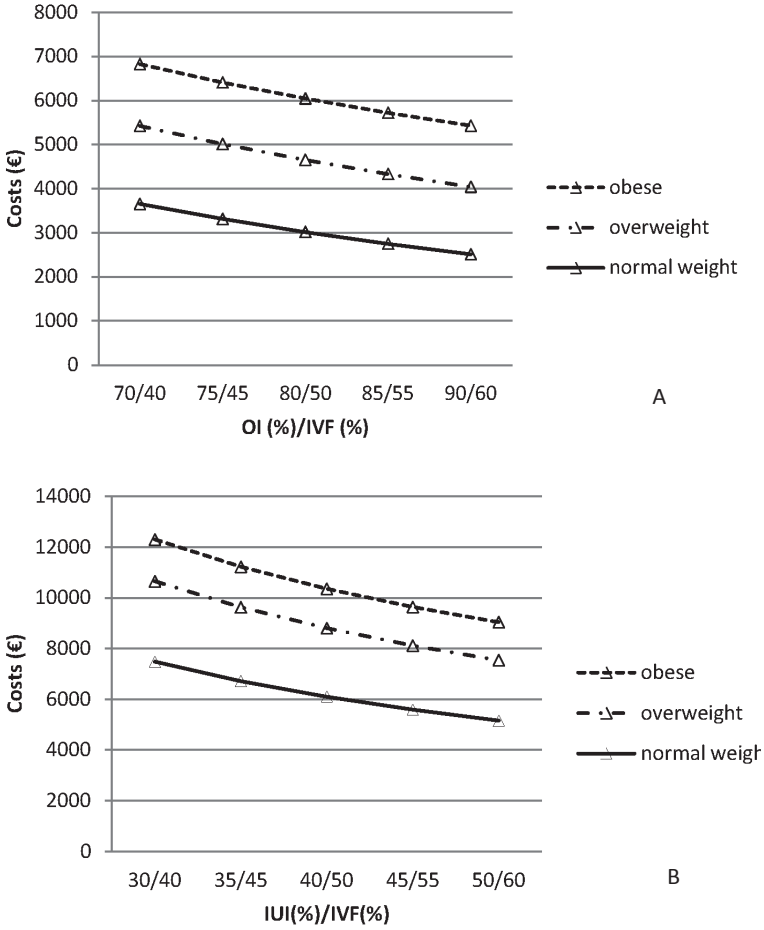


**Figure 1 (A).** Sensitivity analyses showing the effect of variation of success rates of OI and IVF on costs per live birth in anovulatory women. (**B).** Sensitivity analyses showing the effect of variations of success rates of IUI and IVF on costs per live birth in ovulatory women.

Of these pregnancies 78 are expected to suffer a miscarriage and 702 women will have an ongoing pregnancy. The expected number of pregnancies complicated by pre-eclampsia, gestational diabetes and caesarean delivery, will be 70, 35 and 70, respectively, whereas 4 women will suffer stillbirth. Overall, 698 women are expected to deliver a child, for a total cost of € 4258 per woman, resulting in a cost per live birth of € 6096.

**Table 6.** Hypothetical cohort of 1000 ovulatory women in different weight categories

| | Normal weight | Over-weight[a] | Obese[b] |
|---|---|---|---|
| Cohort | 1000 | 1000 | 1000 |
| Impact of overweight on spontaneous pregnancies (OR) | 1 | 1 | 0.9 |
| Spontaneous pregnancies (baseline rate 10%) | 100 | 100 | 90 |
| Number of women undergoing IUI | 900 | 900 | 910 |
| Treatment effect of IUI (OR) | 0.4 | 0.4 | 0.4 |
| Pregnant after 3 cycles IUI (baseline rate 40%) | 360 | 360 | 364 |
| Number of women undergoing IVF | 640 | 640 | 636 |
| Impact of overweight on effectiveness IVF | 1 | 0.71 | 0.68 |
| Pregnant after 2 cycles IVF (baseline rate 50%) | 320 | 227 | 216 |
| Expected number of pregnancies | 780 | 687 | 670 |
| Impact of weight on miscarriage (OR) | 1 | 2 | 2 |
| Expected number of miscarriages (baseline rate 10%) | 78 | 137 | 134 |
| Number of women without ongoing pregnancy | 298 | 450 | 464 |
| Number of women with ongoing pregnancy | 702 | 550 | 536 |
| Impact of weight on pre-eclampsia (OR) | 1 | 2.3 | 3.7 |
| 10% pregnancies complicated by pre-eclampsia | 70 | 126 | 198 |
| Impact of weight on gestational diabetes (OR) | 1 | 2.1 | 3.6 |
| 5% pregnancies complicated by gestational diabetes | 35 | 58 | 97 |
| Impact of weight on caesarean deliveries (OR) | 1 | 1.5 | 2.1 |
| 10% pregnancies caesarean delivery | 70 | 82 | 113 |
| Impact of weight on stillbirth (OR) | 1 | 1.5 | 2.1 |
| 0.5% pregnancies stillbirth | 4 | 4 | 6 |
| Total women with live birth | 698 | 546 | 531 |
| Total costs complications (€) | 867 | 1410 | 2103 |
| Total expected cost (*€1000) | 4258 | 4801 | 5494 |
| Cost per live birth (€) | 6096 | 8800 | 10355 |
| Cost per pregnancy (€) | 6066 | 8734 | 10246 |

[a] Applied BMI (kg/m²) threshold differed from study to study (range 25–27).
[b] Applied BMI (kg/m²) threshold differed from study to study (range 29–35).

Similarly as for anovulatory women, it can be shown that in overweight ovulatory women the effectiveness of treatment decreases, resulting in a decrease of the number of pregnancies and live births, an increase in costs and a relative increase of the number of complications. This results in a decrease in the number of live births of 153 (22%), with the expected increase in costs of € 543 (13%), resulting in a cost per live birth of € 8800. For obese ovulatory women, these figures are worse with a decrease in live births of 167 (24%), with the expected increase in costs of almost € 1250 (29%), resulting in a cost per live birth of € 10,355.

## DISCUSSION

Overweight and obesity are an increasing problem in Western society. In this review, we collected data on the impact of overweight and obesity on fertility care. We found that both in ovulatory and in anovulatory subfertile women overweight and obesity resulted in a decreased fecundity and in an increase in the number of pregnancy complications and associated costs. However, there is no proven cause and effect between overweight and subfertility. It remains possible that excessive weight and subfertility are both symptoms of an unknown pathology.

Our results roughly suggest that overweight leads to an additional cost of € 1500 per pregnancy and 100 fewer pregnancies per 1000 anovulatory women undergoing fertility treatment, where this is € 2500 and 150 pregnancies, respectively, for anovulatory women.

The validity of our findings depends on the robustness of our methodology. We put forward a framework that can be used to encourage development of more advanced models for generating cost-effectiveness information through robust economic evaluation. In our review of the literature, we found different and occasionally conflicting results on the impact of overweight and obesity on the effect of fertility treatment. When this was the case, we chose to consider no effect of overweight. Furthermore the success rate of IVF decreases with increasing BMI (Maheshwari *et al*., 2007), thus overweight and obese women will have to undergo more cycles compared to normal-weight women.

As a consequence, our findings may be an underestimation of the impact of overweight and obesity. Since the purpose of this review was not to give exact figures on costs but to show a trend in costs and cost-effectiveness, we feel this possible inaccuracy does not undermine the overall conclusion.

From our analysis several issues rise. First, as a higher BMI is associated with more pregnancy complications, there is the question as to whether women should lose weight before fertility treatment is started. A recent retrospective analysis by Maheshwari *et al*. (2009) concludes that cost of IVF is not different for several weight categories but because of obstetric complications associated with higher BMI women with overweight should be advised to lose weight prior to IVF. Our analysis concurs with this conclusion and gives indicative results that merit consideration in counselling patients and guiding evidence-based discussions on current practice and policy.

Weight loss may be achieved by lifestyle modification interventions, incorporating multiple approaches (diet, exercise, behaviour). Interventions of this kind are advised as a key component for the improvement of reproductive function in overweight women, specifically with PCOS (Kiddy *et al*., 1992; Clark *et al*., 1995; Huber-Buchholz *et al*., 1999; Hoeger *et al*., 2004; Norman *et al*., 2004; Balen *et al*., 2006; Tang *et al*., 2006), although the evidence of its effectiveness as demonstrated in clinical studies is limited. The

cost-effectiveness of losing weight has never been assessed in large groups of subfertile women with respect to increasing treatment success for weight-related subfertility, prevention of complications during pregnancy and improvement of perinatal outcome. Until this has been demonstrated we do not think it should be obligatory for overweight subfertile women to undergo a lifestyle intervention programme before starting fertility treatment but in counselling patients there should be attention for possible pregnancy complications with increasing BMI. It is clear that losing weight takes great effort and we feel that overweight should be considered a disease rather than an amenable condition.

Second, apart from the unproven effectiveness of lifestyle interventions in overweight subfertile women, there is the question as to whether there should be upper limits for BMI above which couples should not be treated. Some authors have suggested limits for BMI for women undergoing fertility treatment, both with the arguments of patient safety concerns, as well as a lack of effectiveness of treatment of obese women (Gillett *et al*., 2006; Zachariah *et al*., 2006; Maheshwari *et al*., 2007). However, we feel that from the perspective of effectiveness of treatment, our data show that there is no reason to withhold treatment. Although effectiveness rates decrease with increasing BMI, the same appears true for women undergoing assisted reproduction over the age of 40, which is a well accepted practice in many countries. Age is however a predictable and amenable factor considering the fact that many couples delay conception to for example pursue career opportunities. In our opinion, studies on weight loss interventions should show a clear increase of effectiveness of fertility treatment and a clear decrease in pregnancy-related complications, before BMI thresholds can be implemented. In conclusion, in ovulatory and anovulatory subfertile women overweight and obesity is associated with a decrease in the number of pregnancies, a sharp increase in the number of complications with an additional rise of associated costs per pregnancy. There is not enough evidence however to prove that losing weight will improve the outcome of fertility treatment and decrease complications in pregnancies, and therefore strict BMI thresholds cannot be recommended yet. However, overweight and obese subfertile women should be counselled that overweight is a risk factor in pregnancy and is associated with several complications in both mothers and their children.

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Chapter 3

# The subcutaneous abdominal fat and not the intra-abdominal fat compartment is associated with anovulation in women with obesity and infertility

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## ABSTRACT

***Context***: Abdominal fat contributes to anovulation.
***Objective***: We compared body-fat distribution measurements and their contribution to anovulation in obese ovulatory and anovulatory infertile women.
***Design***: 17 ovulatory and 40 anovulatory women (age 30±4 years; body mass index (BMI) 37.7±6.1 kg/m$^2$) participated. Body-fat distribution was measured by anthropometrics, dual-energy x-ray absorptiometry (DEXA) and single-sliced abdominal CT scan (ssCT). Multiple logistic regression analysis was applied to determine which fat compartments significantly contributed to anovulation.
***Results***: Anovulatory women had a higher waist circumference (113±11 vs 104±9 cm; P<0.01), significantly more trunk fat (23.0±5.3 vs 19.1±4.2 kg; P<0.01) and abdominal fat (4.4±1.3 kg vs 3.5±0.9 kg; P<0.05) on DEXA scan than ovulatory women in spite of similar BMI. The volume intra-abdominal fat (IAF) on ssCT was not significantly different between the two groups (203±56 vs 195±71 cm$^3$; P=0.65), but anovulatory women had significantly more subcutaneous abdominal fat (SAF) (992±198 vs 864±146 cm$^3$; P<0.05). After multiple logistic regression analysis, only trunk fat, abdominal fat and SAF were associated with anovulation.
***Conclusions***: Abdominal fat is increased in anovulatory women due to a significant increase in SAF and not in IAF. SAF and especially abdominal and trunk fat accumulation are associated with anovulation.


## INTRODUCTION

The prevalence of obesity in women of child-bearing age ranges from 3% in Africa to almost 25% in Northern America (Haslam and James, 2005) and is expected to increase further. Overweight (BMI 25–29.9 kg/m$^2$) and obesity (BMI≥30 kg/m$^2$) in women have deleterious effects on ovulation frequency with an exponential increase in anovulation with increased body weight (Green *et al*., 1988; Grodstein *et al*., 1994).
Independent of body weight, body-fat distribution, expressed by waist/hip ratio (WHR), has also been shown to influence the reproductive potential. Conception rates, both in spontaneous cycles and in assisted reproduction cycles, decline with accumulation of fat around the waist and trunk, called upper-body obesity (WHR >0.8 in women), independent of body weight (Zaadstra *et al*., 1993; Wass *et al*., 1997). The differential role and contribution of intra-abdominal fat (IAF) and subcutaneous abdominal fat (SAF) compartments to anovulation are not clear. Increased IAF is associated with insulin resistance and the resulting hyperinsulinaemia contributes to anovulation by increased

ovarian androgen secretion (Poretsky, 1991; Dunaif, 1997) leading to lower sex hormone binding globulin (SHBG) concentrations (Pasquali *et al*., 1994; Pasquali *et al*., 2003) and consequently higher free androgen levels. Insulin directly influences intra-ovarian steroidogenesis which may lead to arrest of follicle growth, as has been shown in women with polycystic ovary syndrome (PCOS) (Franks *et al*., 1996; Willis *et al*., 1996).
Increased androgens in turn contribute to upper-body obesity (Escobar-Morreale and San Millan, 2007), thus a vicious circle evolves where androgens favour abdominal fat accumulation and upper-body obesity which in turn facilitates insulin resistance and androgen production (Garg, 2004; Escobar-Morreale and San Millan, 2007). However, many women with upper-body obesity have ovulatory cycles and little or no insulin resistance. The differential role and contribution of the IAF and SAF compartments to insulin resistance and anovulation has not previously been investigated.
There is an ongoing controversy on the relative contribution of the SAF and IAF to insulin resistance and the metabolic syndrome (Lebovitz and Banerji, 2005; Miles and Jensen, 2005). Some studies (Guo *et al*., 1999; Garg, 2004; Miles and Jensen, 2005) indicate that SAF contributes to insulin resistance while others (Lebovitz and Banerji, 2005; Weiss, 2007) to the contrary report that IAF is associated with insulin resistance and the metabolic complications of obesity.

Some studies have shown more trunk- and abdominal fat on DEXA scan in women with PCOS (Hoeger *et al*., 2004; Carmina *et al*., 2007). A study using abdominal ultra-sonography to measure the IAF and SAF showed that non-obese women with PCOS have more IAF compared to a group of lean ovulatory women (Yildirim *et al*., 2003). Another study, using single-slice abdominal CT (ssCT) scan in obese women with PCOS, showed that IAF correlates stronger with insulin resistance than SAF (Lord *et al*., 2006). A cross-sectional study comparing 50 women with PCOS with 28 controls, showed that women with PCOS had significantly more SAF and gluteal subcutaneous fat. However, after adjusting for the differences in BMI and total fat mass, there were no significant differences in IAF and SAF between women with PCOS and controls (Barber *et al*., 2008).
Considering the above-mentioned controversy, the question arises whether the IAF and SAF compartments are different in ovulatory and anovulatory infertile women with obesity, and which compartment, IAF or SAF, is associated with anovulation.
The aim of this study was to assess the contribution of different body-fat distribution measurements, and especially IAF and SAF, to anovulation in women with obesity and infertility.

## SUBJECTS AND METHODS

***Study subjects***

The study was carried out between 2005 and 2008 at the Fertility clinic of the University Medical Center Groningen (UMCG). All infertile women with a BMI>29 kg/m$^2$ who met the following inclusion criteria (infertility ≥1 year, age <38 years, partner with total concentration motile sperm/ejaculate ≥10 million) were approached to participate in a lifestyle intervention programme (Women of Weight with Infertility) in order to achieve weight loss. Of the 60 eligible women that agreed to participate, two did not undergo the baseline assessment due to early drop-out and pregnancy. One anovulatory woman with a BMI of 58 kg/m$^2$ was excluded from the analysis because the ssCT could not be performed accurately due to physical constraints. Except for one anovulatory woman with type 1 diabetes mellitus, no other study subjects were known to have impaired glucose tolerance or type 2 diabetes mellitus. Except for the one subject with type 1 diabetes mellitus receiving insulin therapy, no other study subject received hormonal preparations or metformin in the 3 months preceding inclusion. All women were caucasian, except for one women of asian origin.

Ovulatory status of study subjects was defined as follows:

- women with amenorrhea (cycle interval ≥6 months) were considered anovulatory.
- women with regular cycles ≤42 days underwent an ultrasound monitoring of the menstrual cycle and women were considered ovulatory because follicle development was seen and ovulation was confirmed by a well-timed midluteal progesterone rise >15 nmol/l.
- women with cycles ≤42 days where ovulation could not be identified during ultrasound monitoring as well as women with menstrual cycles between 42 days and 6 months kept a basal body-temperature chart. A midluteal progesterone rise of >15 nmol/l 1 week after a maintained temperature rise was considered indicative of ovulation.

Hyperprolactinaemia, abnormal thyroid function, non-classical 21-hydroxylase deficiency and androgen secreting tumours were excluded in all anovulatory patients.

In all study subjects, the Rotterdam consensus diagnostic criteria for PCOS were considered and subjects were diagnosed as PCOS if two of the following criteria were present: anovulation, hyperandrogenaemia (clinically or biochemically) or polycystic ovary morphology on ultrasound after the exclusion of other endocrine causes of anovulation (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Hirsutism was used a clinical marker of hyperandrogenaemia, and a serum testosterone

level >3.5 nmol/l and a free testosterone level >62 pmol/l was considered as biochemical hyperandrogenaemia.
Informed written consent was obtained from all women, and the study was approved by the Medical Ethical Committee of the UMCG.

***Anthropometric assessment***
The following anthropometric measurements were determined at intake: body weight (kg), height (cm), BMI (= weight (kg) divided by height in meters$^2$), waist circumference (Wc = waist measured at the narrowest part of the torso located between the lower rib and the iliac crest) and WHR. The WHR was calculated by dividing Wc by hip circumference (measured at the level of the greatest gluteal protuberance in a horizontal plane parallel to the floor). All measurements were carried out by the same observer.
A whole body scan was performed by dual-energy x-ray absorptiometry (DEXA), using a Hologic A Discovery Bone Densitometer (Hologic Inc., Bedford, MA, USA). Total fat mass and total fat percentage was determined for the whole body and two regions (trunk and abdominal slice) were specified by computer programming and measured as mentioned in previous studies (Carmina *et al*., 2007).
All participants, positioned supine, underwent a ssCT at the level of the umbilicus (corresponding well with lumbar vertebrae 4-5) at 120-kV exposure. Initially the Siemens Somatom Sensation 16 was used with a total slice thickness of 18 mm (4 subslices x 0.45 cm). During the study a more advanced CT-scan, the Siemens Somatom Sensation 64 was used with a slice thickness of 18 mm (3 subslices x 0.6 cm). Syngo Volume (Siemens, Berlin, Germany) was the software used for both apparatus. To assess the total abdominal fat (TAF) area, each subslice was edited by delineating the total slice with a graph pen, then the adipose tissue volume was computed with an attenuation range of -150 to -50 Hounsfield units. The area for measuring IAF was delineated by drawing a line within de muscle wall surrounding the abdominal cavity en letting the computer program calculate the adipose tissue within this area. SAF area was calculated by subtracting IAF from TAF. These measurements were performed by a single observer. By multiplying the IAF and SAF area (cm$^2$) with each sub-slice (cm) of the ssCT, the IAF and SAF volume (cm$^3$) was calculated and the mean of the subslices recorded for analysis. The edited data were archived in Rogan.

***Biochemical assessment***
All blood samples were taken at intake after an overnight fast of 10 hours. Serum total testosterone was measured by radioimmunoassay, using [1,2,6,7-$^3$H]testosterone as tracer (Amersham Biosciences, Buckinghamshire, UK) and antiserum developed by Pratt (Pratt *et al*., 1978). SHBG was measured with a binding assay using [$^3$H]dihydrotestosterone

(Amersham, Buckinghamshire, UK) and [$^{1}$H]dihydrotestosterone (Sigma-Aldrich, St. Louis, MO, USA). The coefficients of intra-assay variations were 4.9–7.1% for total testosterone and 8.8–10% for SHBG. The interassay variation was 14–19% for total testosterone and 14–17% for SHBG. Reference values for women were 0–3.5 nmol/l for total testosterone and 20−100 nmol/l for SHBG.
Free testosterone was calculated with the formula according to Vermeulen *(Vermeulen et al*., 1999). In the UMCG, the normal range for free testosterone has been estimated at 9–62 pmol/l and values >62 pmol/l are defined as hyperandrogenaemia. For insulin measurement a DSL-1600 Insulin Radioimmunoassay (Diagnostic Systems Laboratories, Inc., Webster, TX, USA) was used. Radio-activity was measured by a gamma-counter. Fasting glucose was determined with a clinical chemistry analyzer. Homeostasis model assessment score for insulin resistance (HOMA-IR), was calculated as [fasting serum insulin (mU/l) x fasting plasma glucose (mmol/l)] / 22.5 by using the digital HOMA calculator which corrects for insulin measurement techniques (Manley *et al*., 2008; Muniyappa *et al*., 2008).

***Statistical analysis***
For comparison between groups, subjects were stratified by ovulatory status into an anovulatory and ovulatory group. Inter-group comparisons were made by using a unpaired Student's *t*-tests for normally distributed continuous variables or a Mann-Whitney *U*-test when the distribution was skewed. Normal distribution was tested using the Kolmogorov-Smirnov test. Multiple logistic regression analyses were /used to determine the contribution of various variables to anovulation after correction for BMI, testosterone and fasting insulin. The goodness of fit of the multiple logistic regression analyses was presented as the Nagelkerke R-square and P-value of the model Chi-square test. All results were considered statistically significant if $P<0.05$.

## RESULTS

***Clinical characteristics of study subjects***
The clinical characteristics of the anovulatory (n=40) and ovulatory women (n=17) are shown in Table 1. The women ranged in age from 20–38 years. The BMI ranged from 29.2–7.4 kg/m$^2$ in the anovulatory women and 29.4–46.3 kg/m$^2$ in the ovulatory women. In 14 of the 17 ovulatory patients, PCOS could be excluded. Three ovulatory patients had a free testosterone level >62 pmol/l; however, due to morbid obesity, the ovaries could not be visualised by transvaginal ultrasonography to exclude polycystic ovary morphology.
In 33 of the 40 anovulatory women PCOS could be diagnosed and in 3 women PCOS could be excluded. In 4 anovulatory women without clinical or biochemical hyperandrogenaemia,

PCOS could not be excluded, because the ovaries could not be visualised by transvaginal ultrasonography due to morbid obesity.

In spite of a non-significant difference in BMI, age and total fat mass on DEXA scan, anovulatory obese women had a significantly higher Wc, WHR, abdominal and trunk fat on DEXA scan and SAF on ssCT compared to the ovulatory obese women. The IAF on ssCT was not significantly different between anovulatory and ovulatory obese women.

Fasting insulin was significantly increased in anovulatory women but the HOMA-IR was not significantly different. Free testosterone was significantly higher in the anovulatory group compared to the ovulatory group mainly due to significantly lower levels of SHBG.

**Table 1.** Clinical characteristics comparing anovulatory to ovulatory obese women with infertility

| Parameter | Anovulatory (n = 40) | Ovulatory (n = 17) | P value |
|---|---|---|---|
| Age (years) | 29.1±4.3 | 31.1±4.1 | 0.09 |
| BMI (kg/m$^2$) | 37.6±4.8 | 35.6±4.9 | 0.15 |
| Waist circumference (cm) | 113±11 | 104±9 | 0.007 [c] |
| Waist/hip ratio | 0.9±0.1 | 0.8±0.1 | 0.02 [b] |
| Total fat DEXA (kg) | 46.0±10.4 | 42.0±10.0 | 0.19 |
| Total % fat DEXA | 41.3±4.6 | 39.9±4.7 | 0.29 |
| Trunk fat DEXA (kg) | 23.0±5.3 | 19.1±4.2 | 0.009 [c] |
| Abdomen fat DEXA (kg) | 4.4±1.3 | 3.5±0.9 | 0.02 [b] |
| TAF ssCT (cm$^3$) | 1199±213 | 1059±187 | 0.02 [b] |
| IAF ssCT (cm$^3$) | 203±56 | 195±71 | 0.65 |
| SAF ssCT (cm$^3$) | 992±198 | 864±146 | 0.02 [b] |
| Testosterone (pmol/l) [a] | 3.9 (1.6–6.5) | 3.1 (1.6–5.4) | 0.09 |
| SHBG (nmol/l) [a] | 23 (8–43) | 32 (9–93) | 0.02 [b] |
| Free testosterone (pmol/l) [a] | 87 (28–217) | 46 (25–131) | 0.009 [c] |
| Fasting glucose (nmol/l) | 4.9±1.2 | 5.1±0.6 | 0.52 |
| Fasting insulin (pmol/l) [a] | 175.8 (25.8–602.7) | 120.1 (43.8–222.4) | 0.046 [b] |
| HOMA-IR [a] | 2.8 (0.5–6.2) | 2.3 (0.8–4.0) | 0.07 |

[a] Data expressed as mean±SD; or as median, range.
[b] Significant at level P≤0.05.
[c] Significant at level P≤0.01.

**Table 2**. Results of multivariate logistic regression analyses of contribution to anovulatory status by different fat measurements in obese women with infertility

| Fat measurements | OR [a] | 95% CI | R-square/P [b] | P [c] |
|---|---|---|---|---|
| Wc (cm) | 0.90 | 0.79 – 1.03 | 0.34 / 0.014 | 0.12 |
| IAF ssCT/100 ($cm^3$) | 1.89 | 0.46 – 7.68 | 0.33 / 0.012 | 0.38 |
| SAF ssCT/100 ($cm^3$) | 0.56 | 0.31 – 1.03 | 0.39 / 0.003 | 0.06 |
| Total body-fat DEXA (kg) | 0.94 | 0.81 – 1.09 | 0.32 / 0.017 | 0.40 |
| Abdominal fat DEXA (kg) | 0.21 | 0.05 – 0.78 | 0.46 / 0.001 | 0.01 |
| Trunk fat DEXA (kg) | 0.73 | 0.54 – 0.99 | 0.42 / 0.002 | 0.04 |

[a] After correction for BMI, fasting insulin and testosterone.
[b] Nagelkerke R-square and P value of model Chi-square test.
[c] Significant at level P≤0.05.



***Contributors of anovulatory status***

To find contributors to the anovulatory status, we performed multivariate logistic regression analysis correcting for BMI, testosterone and fasting insulin (Table 2). Abdominal slice fat and trunk fat on DEXA scan were significant contributors to anovulation, while the contribution of SAF on ssCT to anovulation was of borderline significance (OR 0.56, 95% CI 0.31–1.03, P=0.06). The Nagelkerke R-square on these analyses ranged from 0.33 to 0.4, indicating a reasonable goodness of fit for the prediction of anovulation. The IAF on ssCT, showed no contribution to anovulatory status. Wc as a clinical tool did not significantly predict anovulatory status.

## DISCUSSION

This study indicates that the SAF and not the IAF compartment is significantly increased in anovulatory women with obesity and infertility compared to ovulatory counterparts with similar BMI. Fat accumulation around the trunk and abdomen and the SAF are associated with anovulation, while the IAF compartment is not associated with anovulation.

Previous studies comparing fat distribution in women with PCOS with ovulatory controls also showed more trunk and abdominal fat on DEXA scan in PCOS women even in cases with normal BMI (Puder *et al*., 2005). One study differentiating SAF from the IAF using abdominal ultrasound indicated that women of normal weight with PCOS have more IAF than normal-weight women with regular menstrual cycles (Yildirim *et al*., 2003). After adjusting for the differences in BMI and total fat mass in a study comparing 50 women with PCOS with 28 controls, no significant differences in IAF and SAF were found between the

two groups (Barber *et al*., 2008). Using ssCT in obese women with infertility, our study showed that the IAF is not different between anovulatory and ovulatory women of similar BMI. Considering the difference in mean BMI (36.5 vs <25.0 kg/m$^2$ and 36.5 vs 28.3 kg/m$^2$) between the study populations of the two mentioned studies (Yildirim *et al*., 2003; Barber *et al*., 2008) and the findings of the present study, a possible explanation for the discrepancy in the findings could be the critical intra-abdominal fat threshold hypothesis. According to this hypothesis, with increase in body weight the intra-abdominal fat compartment reaches a point of saturation after which fat is shunted to the subcutaneous fat compartments (Freedland, 2004). This hypothesis could therefore account for increased SAF with increasing BMI. The ratio of the volume of IAF and SAF on ssCT in the present study population was however unchanged across different BMI ranges. On the other hand, the discrepancy of the findings of the present study and previous studies, can also be attributed to the hypothesis of dysregulated upper-body subcutaneous fat (Danforth, 2000; Jensen, 2008). Dysregulated adipocytes of upper-body subcutaneous fat are resistant to the antilipolytical effects of insulin, resulting in the shunting of post-prandial FFAs to the liver and skeletal muscles. This might be mediated by inflammatory changes, increase in adipocyte size and decreased adiponectine synthesis by the subcutaneous fat (Koska *et al*., 2008). Fat storage in the liver and skeletal muscles furthermore contributes to insulin resistance (Guo *et al*., 1999).
Insulin resistance and hyperandrogenaemia are pivotal mechanisms through which obesity and fat distribution leads to anovulation (Dunaif, 1997; Pasquali *et al*., 2003). The finding of significantly higher free testosterone and insulin levels in anovulatory women in the present study supports the findings of previous studies. In the present study, in spite of significantly higher fasting insulin levels, increased insulin resistance in anovulatory women expressed by the HOMA-IR did not reach statistical significance possibly due a small sample size.

The present study supports an association of accumulation of subcutaneous fat around the abdomen and trunk with anovulation and insulin resistance. Increased free fatty acid (FFA) delivery to skeletal muscles seems to be the main mediator of insulin resistance (Boden, 2008). Studies assessing the FFA from different fat compartments show that the subcutaneous fat of the abdomen and trunk (upper body) contribute almost 75% of the total FFAs to the systemic circulation (Guo *et al*., 1999). A simple explanation for the SAF to be the primary cause of insulin resistance is that it is on average 5 times greater in volume than the IAF which only contributes approximately 15% of the total systemic FFAs.
The finding of the present study does however not necessarily exclude functional mechanisms of IAF contributing to anovulation. In spite of a much smaller volume of IAF compared to SAF and much lower contribution of IAF to the systemic FFA concentration,

using multivariate analysis, various studies showed a significant correlation of the IAF with insulin resistance and the metabolic complications of obesity (Lebovitz and Banerji, 2005). IAF has higher lipolytical activity per unit fat mass and direct drainage into the portal vein results in increased delivery of FFAs to the liver (Garg, 2004).
The differential secretion of adipokines and cytokines by the IAF and SAF compartments might also play a contributory role in obesity-related infertility and anovulation. In view of a significantly greater amount of SAF, a different obesity-related mechanism contributing to anovulation could also be considered. Leptin, an adipokine mainly produced by SAF, induces anovulation by direct ovarian effects in animal models (Duggal *et al*., 2000). IAF and SAF compartments are interlinked and changes in the one compartment lead to adaptations in the other (Weiss, 2007). The IAF may contribute more to the pro-inflammatory component of the metabolic syndrome by the secretion of adipokines and cytokines with a different profile compared to subcutaneous fat (ntuna-Puente *et al*., 2008). In women with PCOS and a mean BMI of 35 kg/m$^2$, the IAF showed a stronger correlation with metabolic dysfunction than the SAF (Lord *et al*., 2006). The contributory role of the pro-inflammatory IAF and the voluminous SAF on infertility and anovulation needs further elucidation. The finding of this study only shows an association of the volumes of IAF and SAF with anovulation and no conclusions can be drawn on a cause and effect relationship.
A limitation of this study was the small sample size. Larger studies are needed to confirm our data in obese infertile ovulatory and anovulatory women with respect to body-fat distribution and the role of these different fat compartments in insulin resistance and anovulation. This study was limited to obese women, and cannot be extrapolated to women of normal weight. In 7 of the 57 study subjects PCOS could not be diagnosed or excluded because the ovaries could not be visualised by transvaginal ultrasonography, therefore limiting the comparison of the findings with previous studies on women with PCOS. Excluding the one anovulatory women with type 1 diabetes mellitus would not have changed the outcome of the analysis. Because 56 of the 57 study subjects were from caucasian origin, the presented data cannot be extrapolated to non-caucasian populations.

In conclusion, in anovulatory women with obesity and infertility, the total amount of abdominal and trunk fat is significantly increased, with an significant increase in the volume of SAF and no difference in the volume of IAF compared to ovulatory women with obesity and infertility. Abdominal and trunk fat accumulation and SAF accumulation but not the IAF are associated with anovulation. This association is however only based on the volume of these fat compartments and does not necessarily reflect the consequences of their metabolic activity.

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Chapter 4

# The measurement of intra-abdominal fat by ultrasound correlates well with CT scan in women with obesity and infertility

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*Submitted*

## ABSTRACT

The aim of the study was to investigate the correlation between the measurements of intra-abdominal fat (IAF) and subcutaneous abdominal fat (SAF) by abdominal ultrasound (US) and Computerised Tomography (CT) in women with obesity and infertility. In 53 participants undergoing a lifestyle programme, the Pearson's correlation coefficients were calculated of the measurements of IAF and SAF by CT and US at fixed time points (baseline, month 3 and month 6) and of the changes of IAF and SAF. A method comparison analysis was performed for the IAF measurements. The correlation between the IAF measurement by US and CT was good at all time points (all $r>0.71$). The correlation between the SAF measurement by US and CT was reasonable at baseline ($r=0.54$) and poor at month 3 and month 6 (all $r\leq0.39$). The correlation between the measurement of the changes of IAF by US and CT was only significant between baseline and month 6 (all $r>0.48$). The correlation of the measurement of the changes of SAF between US and CT was poor (all $r<0.23$). The method comparison analyses for the IAF measurements showed good agreement. In conclusion, in women with obesity and infertility, the measurement of IAF by US correlates strongly with the measurement of IAF by CT scan. US can measure the changes of IAF over time only with when sufficient loss of IAF has occurred. The measurement of SAF by US is unreliable and requires further methodological improvement.




## INTRODUCTION

Increased accumulation of fat around the abdomen, called abdominal obesity, contributes strongly to the metabolic consequences of obesity on health and it plays a role in female infertility and pregnancy complications (Despres *et al*., 2001; McCarthy *et al*., 2004; Haslam and James 2005; Nelson and Fleming, 2007; Brisson *et al*., 2010;). Waist circumference (Wc) measurement is used to identify individuals with abdominal obesity (Janssen *et al*., 2002; Zhang *et al*., 2008); however, it cannot differentiate between intra-abdominal fat (IAF) and subcutaneous abdominal fat (SAF) accumulation (Despres, 2006; Jensen, 2008; Weiss, 2007). The reliable measurement of IAF and SAF is not only important as a tool to predict cardiovascular and metabolic disease risk, but it is also essential to evaluate the effect of these fat compartments on female reproductive function. IAF accumulation is related to insulin resistance in women with Polycystic Ovary Syndrome (PCOS) (Lord *et al*., 2006) and in these women the resulting hyperinsulinaemia contributes to anovulation (Pasquali *et al*., 2006). Obese anovulatory women with PCOS who resume ovulation during a 6-month lifestyle programme lose more IAF with no

difference in the change of SAF compared to the women who did not resume ovulation (Kuchenbecker *et al*., 2011). On the other hand, in women with obesity and infertility, SAF and not IAF was associated with anovulation after correcting for BMI, insulin and testosterone (Kuchenbecker *et al*., 2010). Increased IAF during early pregnancy is associated with insulin resistance and increased diastolic blood pressure (Bartha *et al*., 2007) and it can predict glucose intolerance in later pregnancy (Martin *et al*., 2009).
Considering the worldwide obesity epidemic and its consequence on female reproduction, more studies are needed on the influence of the changes of IAF and SAF on female reproduction. Abdominal ultrasound (US) might be a good tool for the measurement of IAF and SAF due to the absence of radiation exposure, low cost and its general availability in fertility and antenatal clinics.
Several studies in various populations have confirmed that US is a reliable tool for the measurement of IAF and SAF when compared to the gold standard of Abdominal Computerised Tomography scan (CT) or Magnetic Resonance Imaging (MRI) (Armellini *et al*., 1993; Stolk *et al*., 2001; De Lucia Rolfe *et al*., 2010; Gradmark *et al*., 2010). However, these validation studies were mostly conducted in older populations and have not been performed in women of reproductive age before. It is relevant to do so, since body composition and abdominal fat distribution change with age, potentially affecting the reliability of the US measurement technique. Furthermore, only one study tried to validate the measurement of the changes of IAF and SAF between US and CT in study subjects undergoing weight loss (Armellini *et al*., 1991). Before introducing US as a tool for studying the effect of the changes of IAF and SAF on the reproductive outcome in women undergoing lifestyle intervention, validation of the US technique by comparing CT and US measurement is required in obese women of reproductive age.

The first aim of this methodological study was to investigate the correlation and agreement of the measurement of IAF and SAF between US and CT in women with obesity and infertility at fixed time points (baseline, month 3 and month 6) of a lifestyle programme to achieve weight loss. The second aim of the study was to evaluate the correlation of the measurement of the changes of IAF and SAF between US and CT between baseline and month 3 and baseline and month 6 of the lifestyle programme.

## METHODS AND PROCEDURES

***Subjects***

Participants were women with obesity and infertility attending the Fertility clinic of the University Medical Center Groningen (UMCG) between 2005 and 2008. All women with a BMI >29 kg/m$^2$ who met the inclusion criteria (infertility ≥1 year, age <38 years, partner with total motile sperm concentration/ejaculate ≥10 million) were approached to participate in a lifestyle programme. Informed written consent was obtained and the study was approved by the Medical Ethics Committee of the UMCG. The lifestyle programme comprised dietary intervention, increased physical activity and behaviour modification as described before (National Heart, Lung and Blood Institute/National Institutes of Diabetes and Digestive and Kidney diseases, 1998; Kuchenbecker *et al*., 2011).



Anthropometric measurements and US and CT measurements of IAF and SAF were performed at baseline, at month 3 and month 6 of the lifestyle programme. At these three respective time points all measurements of each patient were performed on the same day. Pregnancy was excluded before each CT. For this analysis, 53 participants for whom both the US and CT data were available were selected from the total cohort of 57 women undergoing the lifestyle programme as published previously in (Kuchenbecker *et al.*, 2010).

***Anthropometric measurements***

Body weight (up to nearest 0.1 kg) and height (up to nearest 1 cm) were measured using a calibrated scale and stadiometer (SECA model 764, Seca, Birmingham, UK) with participants wearing light indoor clothes and no shoes. BMI was calculated as weight in kg divided by height in square meters (m$^2$). Waist circumference (Wc) was measured (up to the nearest 0.5 cm) at the narrowest part of the torso located between the lower rib and the iliac crest using a CEFES®-CONTROL tape measure (HOECHTMASS Balzer GmBH, Sulzbach, Germany).

Using a Harpenden Skinfold Caliper (Baty International, Burgess Hill, West Sussex , UK), four skinfold thickness (SFT) measurements were performed in duplicate on the right side of the body with all subjects in a standing position. The SFT measurements were performed according to the Anthropometric Standardization Reference Manual (Lohman and Roche, 1991) and included the triceps, biceps, subscapular and supra-iliac measurements and the total sum of the four skinfolds was calculated.

***Abdominal Ultrasound (US)***

US was performed using a ALOKA SSD-1000 (Aloka, Tokyo, Japan) ultrasound machine with a 3.5 MHz convex-array abdominal transducer to measure IAF and SAF. A validated

protocol was followed to perform the measurements (Stolk *et al*., 2001). In short, all measurements were performed at the level of the Wc. To avoid distorting of the abdominal cavity, all measurements were taken at the end of a quiet expiration. IAF measurements were performed in the midline and the right and left longitudinal lines (10 cm to the left and right of the midline, respectively) in a longitudinal plane. IAF was measured as the distance in cm from the anterior boundary of the lumbar vertebra and the peritoneal boundary of the anterior abdominal wall. SAF was measured in the midline, in a transversal plane at the level of the Wc, as the distance in cm from the cutaneous boundary to the linea alba.

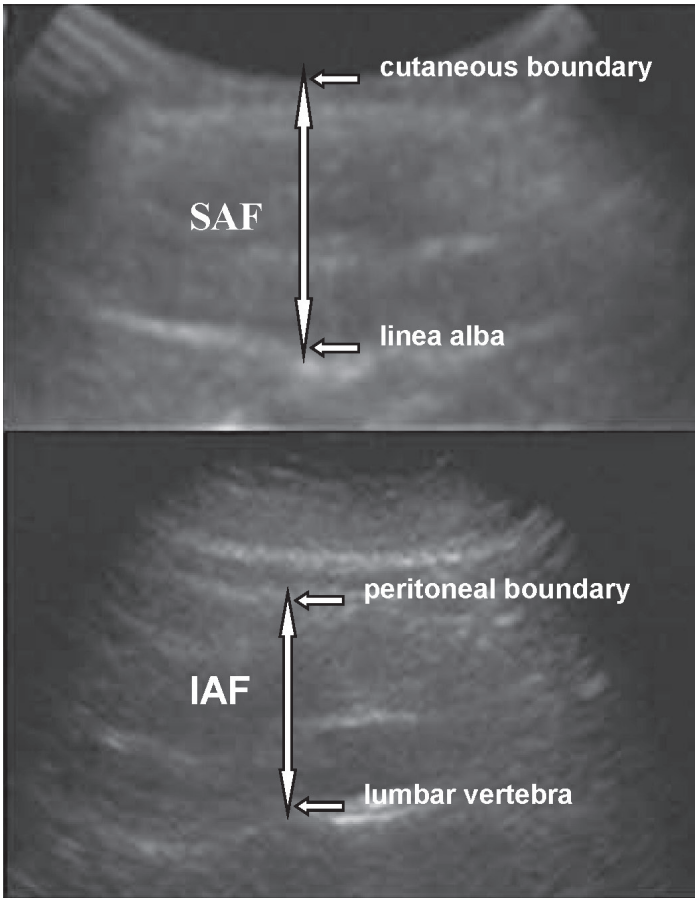


**Figure 1.** Example of an ultrasound (US) showing the intra-abdominal fat (IAF) and the subcutaneous abdominal fat (SAF) measurements.

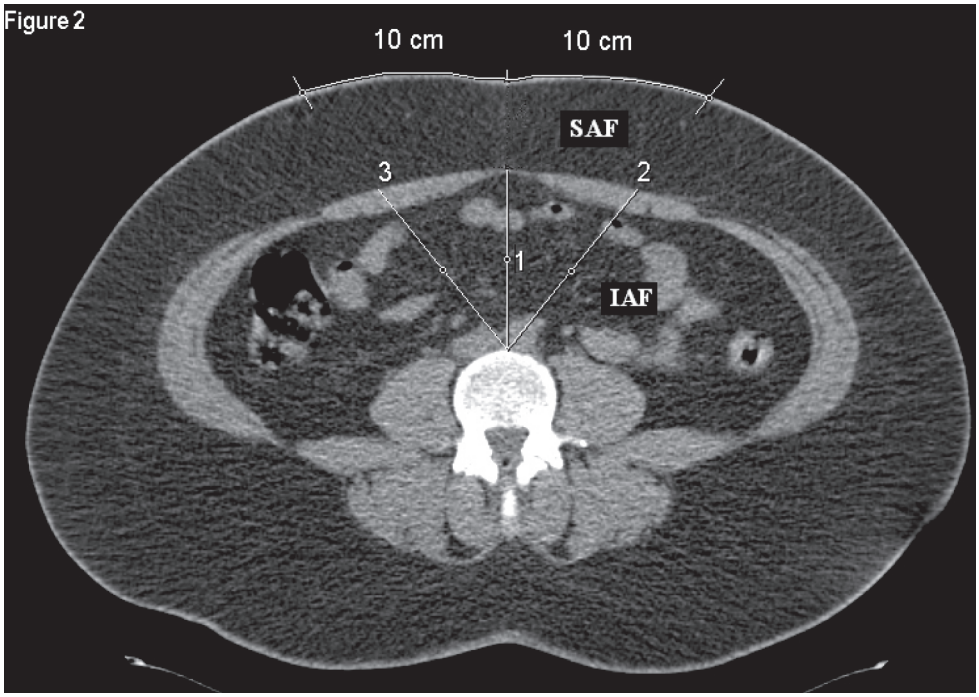


**Figure 2.** Example of a CT scan showing the intra-abdominal fat (IAF) and the subcutaneous abdominal fat (SAF). The lines 1, 2 and 3 indicate the location of the distance measurement of IAF midline, left and right, respectively.

The image was captured when the transducer just had contact with the skin to avoid compression of the subcutaneous adipose tissue. See Figure 1 for an illustration of the US measurements of IAF and SAF. All measurements were obtained by a single trained observer, blinded to the measurements by CT. The data are presented in cm (to the nearest 0.1 cm) as SAF midline, IAF midline, IAF midline and right and IAF mean (mean of midline, right and left). The left measurement of IAF was often difficult to obtain due to reflection by gas in the descending colon. We therefore evaluated IAF by US with and without the measurement of the left longitudinal plane separately. The intra-observer variability was previously shown to be 1.8–2.9% for IAF and 0.6–3.0% for SAF (De Lucia Rolfe *et al.*, 2010)

***CT scan***

A single-sliced abdominal CT scan with a total slice thickness of 18 mm (consisting of 3–4 subslices) was performed at the level of the umbilicus (corresponding with lumbar vertebrae 4-5) and the measurement of the IAF and the SAF volume ($cm^3$) was calculated as published previously by us (Kuchenbecker *et al*., 2010). For the method comparison analysis, IAF was measured on the most cranial subslice of the CT scan as a distance in cm from the anterior boundary of the lumbar vertebra and the peritoneal boundary of the anterior abdominal wall in the midline and 10 cm to the left and right of the midline, respectively. The data are presented in cm (to the nearest 0.1 cm) as IAF midline, IAF midline and right and IAF mean (mean of midline, right and left). Distance measurement of the SAF was not possible due the distortion of SAF by the umbilicus in the midline of the CT scans. See Figure 2 for an illustration of the distance measurements of IAF. Although distance measurement of IAF on the CT scan is inferior to volume measurement, the distance measurement was necessary for the Blant-Altman plot which requires similar units of measurement for both methods. All measurements were performed by a single observer blinded for the results of the US measurements. The edited data were archived in Rogan.

***Statistical analysis***

Data were expressed as mean±standard deviation. Pearson's correlation coefficients were used for comparison of the methods. Cross-sectional comparisons were made at fixed time points (baseline, month 3 and month 6) of the lifestyle programme. For longitudinal comparison, the changes between baseline and month 3 and baseline and month 6 as well as the percentage of change were calculated. Method comparison analysis was performed by constructing Bland-Altman plots to evaluate the extent to which the IAF measurement by US and CT agreed. All analyses were performed using SPSS, versions 17 and 18 (SPSS Inc., Chicago, IL, USA). A P value of $<0.05$ was considered statistically significant.

## RESULTS

The baseline data of the 53 included women (mean BMI 37.0±4.9 $kg/m^2$) are presented in Table 1. At month 3 and month 6 of the lifestyle programme, 35 and 27 participants remained for analysis due to pregnancy and drop-out (11 pregnant and seven drop-outs at month 3; one pregnant and seven additional drop-outs at month 6). The 6-month lifestyle programme resulted in considerable weight loss (mean -6.0 %; range 6.25% to -24.4%).

**Table 1.** Measurements in women with obesity and infertility undergoing a lifestyle programme

| Method | Baseline (n=53) | Month 3 (n=35) | Month 6 (n=27) | Month 3 (n=24) | Month 6 (n=24) |
|---|---|---|---|---|---|
| | | | | Completers data | |
| Age (years) | 29.7±4.3 | | | | |
| BMI (kg/m$^2$) | 37.0±4.9 | 36.6±4.9 | 35.2±5.5 | -1.1% | -4.9% |
| Body weight (kg) | 106.2±15.5 | 106.7±14.5 | 102.9±16.2 | -3.3% | -6.0% |
| Waist circumference (cm) | 110±11 | 109±13 | 107±14 | -0.9% | -2.6% |
| **US** | n=53 | n=33 | n=24 | | |
| SAF (cm) midline | 5.4±1.2 | 5.3±1.0 | 5.2±1.0 | -0.6% | -1.6% |
| IAF (cm) midline | 7.4±2.0 | 7.1±1.9 | 6.4±1.9 | -10.0% | -14.7% |
| IAF (cm) midline and right | 7.9±2.0 | 7.6±1.9 | 6.9±1.8 | - 9.9% | - 12.6% |
| IAF (cm) mean | 7.8±1.9 | 7.7±2.0 | 6.9±1.9 | - 10.1% | - 13.0% |
| **CT** | n=53 | n=35 | n=27 | | |
| SAF CT scan (cm$^3$) | 956±198 | 929±158 | 896±208 | -2.8% | -6.3% |
| IAF CT scan (cm$^3$) | 202±62 | 197±56 | 182±69 | -2.5% | -9.8% |
| IAF midline (cm) | 8.2±2.2 | 8.3±2.2 | 7.4±2.0 | -0.3% | -9.2% |
| IAF midline and right (cm) | 8.9±2.1 | 9.0±2.0 | 8.3±1.8 | -0.2% | -8.6% |
| IAF mean (cm) | 9.1±2.0 | 9.2±2.0 | 8.5±1.7 | -0.2% | -6.1% |
| **SFT** | n=53 | n=35 | n=27 | | |
| Biceps (mm) | 29.3±5.8 | 27.8±6.5 | 27.0±7.4 | -5.7% | -6.9% |
| Triceps (mm) | 32.9±6.3 | 32.9±6.2 | 31.5±6.3 | -0.6% | -1.1% |
| Subscapular (mm) | 38.9±5.4 | 37.2±7.0 | 34.2±9.1 | -6.9% | -13.9% |
| Supra-iliacal (mm) | 37.7±6.9 | 36.9±6.9 | 33.7±6.0 | -4.7% | -10.7% |
| Sum of total (mm) | 138.7±16.9 | 134.0±20.3 | 128.7±26.6 | -6.2% | -9.8% |

Data are presented as mean±SD and % change from baseline; percentages presented are based on completers data. BMI, body mass index; CT, abdominal CT scan; IAF, intra-abdominal fat; SAF, subcutaneous abdominal fat; SFT, skinfold thickness; US, abdominal ultrasound.

The mean loss of SAF measured by US between baseline and month 6 was small (mean -1.6%; range 23.7% to -28.1%) compared to the mean loss of SAF measured by CT (mean -6.3%; range 32.9% to -26.2%). Measurement of the loss of IAF by US between baseline and month 6 (mean -13.2%; range 15.3% to -36.5%) was in agreement with the volume measurement by CT (mean -9.8%; range 18.6% to -20.9%).

Table 2 shows that the US measurements of IAF and SAF correlated well with the CT measurements of IAF (range r=0.739 to r=0.797) and SAF (r=0.538) at baseline. At month 3 and month 6 the correlation between the US and CT measurement of SAF was poor (r=0.390 and r=0.328, respectively). The correlations between the measurement of IAF by US and CT remained strong (range r=0.716 to r=0.898) at 3 and 6 months. The median BMI of the total group (36.5 kg/m$^2$) was chosen to select two BMI groups for a sub-

analysis. The correlation of the IAF measurements between US and CT at baseline were comparable in participants with a BMI ≥36.5 kg/m$^2$ (range r=0.659 to r=0.758) as compared to a BMI <36.5 kg/m$^2$ (range r=0.738 to r=0.753). The correlation of the SAF measurement between US and CT was however less accurate (r=0.242) in participants with a BMI≥ 36.5 kg/m$^2$ compared to those with a BMI <36.5 kg/m$^2$ (r=0.590). The comparison of method analysis between the measurement of IAF by US and CT in distance at baseline showed a mean negative bias for IAF of -1.1 (95% limits of agreement: -3.9 to 1.6) (Figure 3).

Changes in IAF and SAF were recorded between baseline and month 3, and baseline and month 6, using US and CT. The change in SAF over time measured by US did not correlate with the change measured by CT (Table 3). To exclude the possibility that loss of SAF was too little to measure the change of SAF accurately, skin-fold thickness (SFT) measurement was added. The sum of the SFT measurement showed comparable loss of SAF between SFT and CT (Table 1) and a significant correlation between the CT and SFT measurements of SAF at month 3 (r=0.347) and month 6 (r=0.432).

The correlation between the changes of IAF measured by US and CT was poor between baseline and month 3 (range r=0.238 to r=0.298) and significant (range r=0.486 to r=0.580) between baseline and month 6 (Table 3). The comparison of method analysis between the measurement of the changes of IAF by US and CT in distance between baseline and month 6 showed a mean negative bias for IAF of -0.2 (95% limits of agreement: -2.1 to 1.7) (Figure 4).

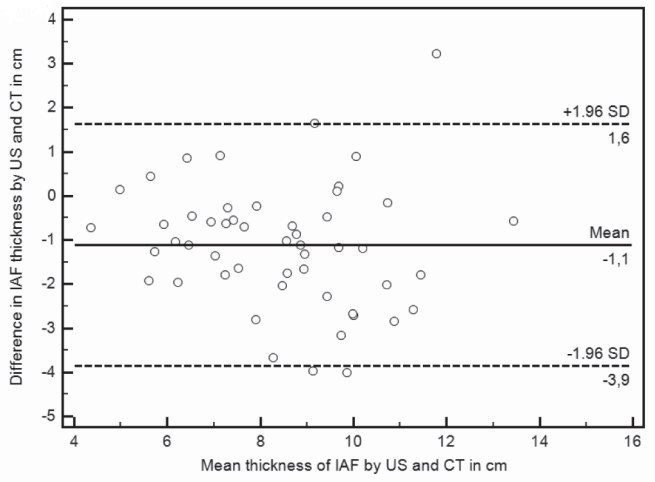


**Figure 3.** Bland-Altman plot representing the mean intra-abdominal fat (IAF) thickness in cm by ultrasound (US) and CT scan (CT) on the x-axis and the difference between the IAF measurement by CT and US in cm on the y-axis.

**Table 2.** Correlations of measurements of SAF and IAF at fixed time points during a lifestyle programme

| | Baseline (n=53) | | | Month 3 (n=33) | | | Month 6 (n=24) | | |
|---|---|---|---|---|---|---|---|---|---|
| | r | $R^2$ | P value | r | $R^2$ | P value | r | $R^2$ | P value |
| **SAF by US in cm** | | | | **SAF by CT $cm^3$** | | | | | |
| SAF midline | 0.538 | 0.289 | <0.001 | 0.390 | 0.152 | 0.020 | 0.328 | 0.107 | 0.13 |
| **Subcutaneous fat by SFT in mm** | | | | **SAF by CT $cm^3$** | | | | | |
| Biceps | 0.261 | 0.068 | 0.052 | 0.455 | 0.207 | 0.005 | 0.381 | 0.145 | 0.050 |
| Triceps | 0.414 | 0.171 | 0.002 | 0.508 | 0.258 | 0.001 | 0.734 | 0.539 | <0.001 |
| Subscapular | 0.441 | 0.194 | 0.001 | 0.556 | 0.309 | <0.001 | 0.582 | 0.339 | 0.001 |
| Supra-iliacal | 0.292 | 0.047 | 0.029 | 0.327 | 0.107 | 0.048 | 0.713 | 0.509 | <0.001 |
| Sum of total | 0.507 | 0.257 | <0.001 | 0.596 | 0.355 | <0.001 | 0.751 | 0.564 | <0.001 |
| Biceps | 0.261 | 0.068 | 0.052 | 0.455 | 0.207 | 0.005 | 0.381 | 0.145 | 0.050 |
| **IAF by US in cm** | | | | **IAF by CT in $cm^3$** | | | | | |
| IAF midline | 0.739 | 0.546 | <0.001 | 0.716 | 0.513 | <0.001 | 0.898 | 0.807 | <0.001 |
| IAF midline and right | 0.749 | 0.561 | <0.001 | 0.731 | 0.534 | <0.001 | 0.898 | 0.807 | <0.001 |
| IAF mean | 0.797 | 0.635 | <0.001 | 0.727 | 0.529 | <0.001 | 0.894 | 0.806 | <0.001 |
| **IAF by CT in cm** | | | | **IAF by CT in $cm^3$** | | | | | |
| IAF midline | 0.781 | 0.610 | <0.001 | 0.786 | 0.618 | <0.001 | 0.815 | 0.664 | <0.001 |
| IAF midline and right | 0.789 | 0.622 | <0.001 | 0.791 | 0.625 | <0.001 | 0.817 | 0.667 | <0.001 |
| IAF mean | 0.794 | 0.631 | <0.001 | 0.791 | 0.625 | <0.001 | 0.825 | 0.680 | <0.001 |

$r$ = Pearson correlation coefficient; $R^2$ = adjusted R square; significant at level $P < 0.05$.
CT, abdominal CT scan; IAF, intra-abdominal fat; SAF, subcutaneous abdominal fat; SFT, skinfold thickness; US, abdominal ultrasound.

**Table 3.** Correlations of the measurements of changes in SAF and IAF during a lifestyle programme

| | Month 3 vs Baseline (n=33) | | | Month 6 vs Baseline (n=24) | | |
|---|---|---|---|---|---|---|
| | r | $R^2$ | P value | r | $R^2$ | P value |
| **SAF by US in cm** | | | **SAF by CT cm$^3$** | | | |
| SAF midline | 0.215 | 0.046 | 0.221 | 0.229 | 0.052 | 0.305 |
| **Subcutaneous fat by SFT in mm** | | | **SAF by CT cm$^3$** | | | |
| Biceps | 0.274 | 0.075 | 0.106 | 0.256 | 0.066 | 0.197 |
| Triceps | 0.030 | 0.000 | 0.860 | 0.531 | 0.282 | 0.004 |
| Subscapular | 0.297 | 0.088 | 0.083 | 0.190 | 0.036 | 0.342 |
| Supra-iliacal | 0.121 | 0.015 | 0.481 | 0.077 | 0.006 | 0.704 |
| Sum of total | 0.347 | 0.12 | 0.041 | 0.432 | 0.187 | 0.024 |
| **IAF by US in cm** | | | **IAF by CT in cm$^3$** | | | |
| IAF midline | 0.298 | 0.089 | 0.087 | 0.486 | 0.216 | 0.022 |
| IAF midline and right | 0.238 | 0.057 | 0.231 | 0.538 | 0.177 | 0.012 |
| IAF mean | 0.240 | 0.057 | 0.187 | 0.580 | 0.252 | 0.007 |
| **IAF by CT in cm** | | | **IAF by CT in cm$^3$** | | | |
| IAF midline | 0.566 | 0.321 | <0.001 | 0.509 | 0.259 | 0.008 |
| IAF midline and right | 0.587 | 0.345 | <0.001 | 0.480 | 0.231 | 0.013 |
| IAF mean | 0.593 | 0.352 | <0.001 | 0.493 | 0.243 | 0.010 |

r, Pearson correlation coefficient; $R^2$, adjusted R square; significant at level P<0.05. CT, abdominal CT scan; IAF, intra-abdominal fat; SAF, subcutaneous abdominal fat; SFT, skinfold thickness; US, abdominal ultrasound.

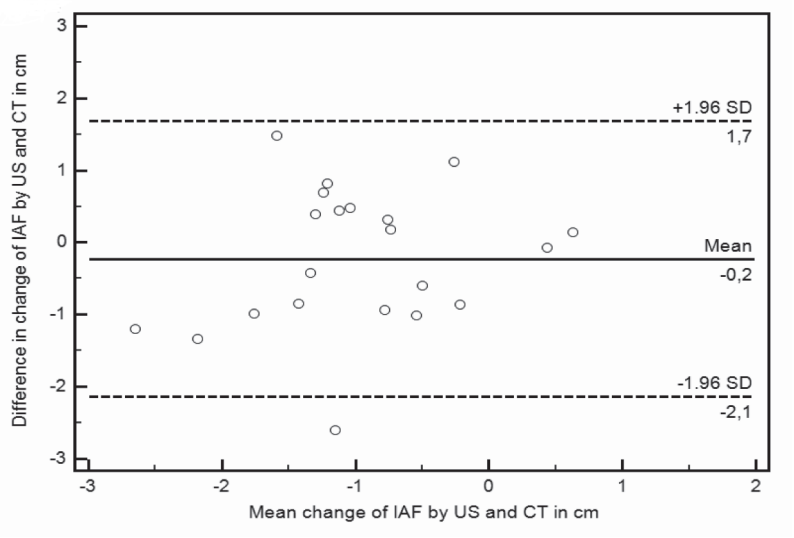


**Figure 4.** Bland-Altman plot representing the mean change intra-abdominal fat (IAF) thickness in cm between baseline and month 6 by ultrasound (US) and CT scan (CT) on the x-axis and the difference between the change of IAF by US and CT in cm on the y-axis.

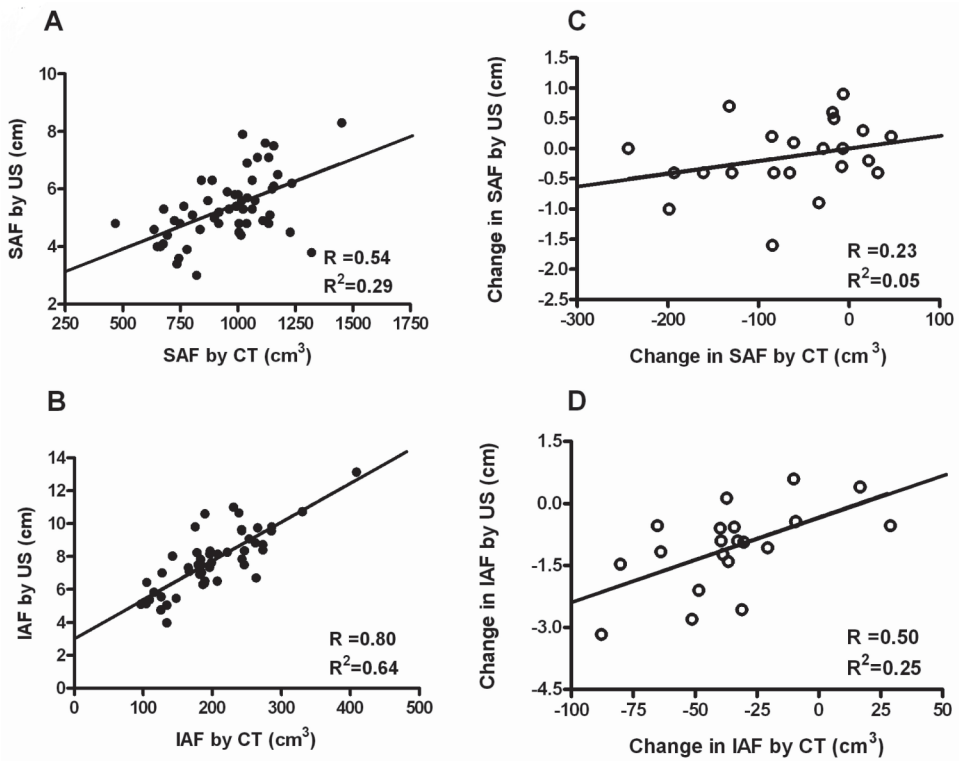


**Figure 5.** (**A**) Correlation between subcutaneous abdominal fat (SAF) by CT scan (CT) and ultrasound (US) at baseline of a lifestyle program. (**B**) Correlation between intra-abdominal fat (IAF) by CT and US at baseline of a lifestyle program. (**C**) Correlation between the changes in SAF measured by CT and US after 6 months. (**D**) Correlation between the changes in IAF measured by CT and US after 6 months. Negative numbers indicate a decrease.

Figure 5 summarises the most important correlations presented in the Tables 2 and 3. Figure 5B in particular, illustrates that the measurement of IAF by US correlated well with the measurement by CT. As shown in Figure 5C and D, the correlation of the changes over time measured by US and CT between baseline and month 6, were stronger for IAF than for SAF.

## DISCUSSION

The most important finding of the present study is that in women with obesity and infertility, the measurement of IAF by US is accurate and comparable to the measurement of IAF by CT. Considering the detrimental role of IAF accumulation on female reproductive function and pregnancy outcome, the measurement of IAF by US can be a valuable tool in reproductive research on IAF.

The correlation found in our study between the measurement of IAF by US and CT at the three fixed time points of the lifestyle programme is comparable to the findings of previous validation studies in different patient populations and different BMI and age groups (Armellini *et al*., 1993; Stolk *et al*., 2001; De Lucia Rolfe *et al*., 2010; Gradmark *et al*., 2010). In agreement with the previous validation studies, we have also shown that the correlation of the measurement of SAF by US and CT is weaker than the measurement of IAF by these two methods (all $r \leq 0.54$ versus all $r > 0.71$). A sub-analysis in participants with different BMI levels (BMI $\geq 36.5$ kg/m$^2$ versus BMI $< 36.5$ kg/m$^2$) revealed that the findings remained unchanged for the measurement of IAF, but that the correlation of the measurement of SAF by US and CT was less accurate in the higher BMI group.

The detection of changes in IAF over time by US compared to the CT measurements, were not as strong as the correlation of the measurements at the fixed time points. An important observation was that the detection of changes in IAF was poor at month 3 and significant at month 6 with 2.5% and 9.8% loss of IAF measured by CT, respectively. This suggests that the use of US for the measurement of the changes in IAF is only reliable when the change in IAF over time is large enough. The comparison of method analysis at baseline, showed a mean negative bias of -1.1 cm of the measurement of IAF by US compared to CT in distance (as a surrogate for the volume measurement) (Figure 3). The limits of agreement were wide and tend to increase with an increase in the distance of IAF. The comparison of method analysis of the measurement of the changes of IAF between baseline and month 6 showed a good agreement between US and CT (Figure 4). Considering the wide range of the limits of agreement between the measurement of IAF by US and CT (Figures 3 and 4), the accuracy of US is not sufficient to be used for diagnostic purposes in individual patients, but may perform well in a cohort-based analysis or in an epidemiological setting to investigate the role of IAF in female reproduction.

In contrast with the findings for IAF, the changes over time in SAF as detected by CT could not be reproduced by US. This could be explained by the fact that US only showed -1.6% loss of SAF compared to -6.1% loss of SAF measured by CT. A sub-analysis (data not presented) of 10 women who lost a mean of 12.7 kg of body weight, also showed that even with greater weight loss, US is not able to detect the changes in SAF reliably. This raises the question, whether the supra-umbilical anatomic location used to measure SAF by US in

the present study, is suitable in obese women of reproductive age. Subcutaneous fat depots are heterogeneous and changes in one depot might not reflect changes in the other depots. Too little loss of SAF as one explanation for the poor performance of US for the measurement of SAF is unlikely, because the sum of SFT measurements also showed comparable loss of subcutaneous fat as measured by CT. We recommend that future studies should investigate whether measurement of SAF by US at multiple locations improves the accuracy of the measurement of the changes of SAF.

Our study confirms the findings of the only previous validation study showing that US can measure changes in IAF but not in SAF compared to CT (Armellini *et al*., 1991). In this study subjects lost on average 1.9 cm of IAF on US compared to 1 cm (over 6 months) of IAF on US in our study. The changes in IAF in this study was measured by CT as change in surface ($cm^2$) compared to change in volume ($cm^3$) as measured in our study. Therefore, the changes in IAF and SAF on CT cannot be compared between these two studies.

The following limitations of this study should be considered. IAF and SAF distribution were captured by a single-sliced abdominal CT scan at the level of L4-L5, whilst the outcome depends on the individual variation of IAF and SAF distribution which can be measured more accurately by whole abdominal CT or MRI scan (Lee *et al*., 2004, Thomas and Bell 2003). Future validation studies should therefore use multiple slices of abdominal CT or MRI to calculate the volume of IAF and SAF. A study published after the present study was launched (Kuk *et al*., 2010), indicates that in women the measurement of IAF by single-sliced CT scan at the level of L2-L3 shows better correlation with whole abdomen CT scan (r=0.95) than single-sliced CT scan at the level of L4-L5 (r=0.89).

It was noted that the left IAF measurement by US was often disturbed and difficult to perform due to gas in the descending colon. This study shows that the left measurement of IAF can be omitted, because the medial, and the mean of the medial and right measurements of IAF still show good correlation with the CT measurement (Tables 2 and 3).

In conclusion, in women with obesity and infertility, there is a strong correlation between the measurements of IAF by US and CT. Furthermore, US can only measure the changes of IAF over time if sufficient loss of IAF has occurred. Considering the safety and general availability of abdominal US machines at fertility clinics and antenatal clinics, the use of US is an affordable tool for further research on the role of IAF on female reproductive function. Measurement of SAF by US proved not to be reliable enough for implementation in its present form and further studies are required to investigate whether measurement of SAF at other anatomic locations will improve its accuracy as compared to CT.

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Chapter 5

# Polycystic ovary syndrome does not determine the association between serum adipokine levels and body-fat distribution in women with obesity and infertility

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## ABSTRACT

***Objective*:** Body-fat distribution and the adipose tissue function and morphology is different in anovulatory obese women with polycystic ovary syndrome (PCOS) compared to ovulatory non-PCOS controls. The aim of this study was to assess whether PCOS status (i.e., defined as anovulation and polycystic ovaries on ultrasound and/or hyperandrogenaemia) is a determining factor in the association between body-fat distribution parameters and serum adipokine levels in women with obesity and infertility.
*Design*: Prospective cohort study
***Methods*:** The differences in body mass index (BMI), waist circumference, total-, trunk- and abdominal fat on DEXA scan and intra-abdominal fat (IAF) and subcutaneous fat (SAF) on single-sliced CT scan and serum adipokine levels (leptin, adiponectin, interleukin-6 and tumour necrosis factor $\alpha$) were assessed in 32 women with anovulatory PCOS and 15 ovulatory non-PCOS controls (mean BMI of total group 36.8±4.9 kg/m$^2$). The correlation between body-fat distribution parameters and serum adipokine levels was assessed followed by linear regression analysis correcting for PCOS status, BMI and age. Liver-fat accumulation was assessed by abdominal ultrasound.
***Results***: Women with anovulatory PCOS had significantly more abdominal obesity (waist circumference, trunk fat – and abdominal fat on DEXA scan) and significantly more SAF. Abdominal ultrasound revealed a trend of more moderate to severe liver-fat accumulation in anovulatory PCOS women compared to ovulatory non-PCOS controls. Leptin showed a significant positive correlation with SAF, trunk, abdominal fat and total fat on DEXA scan and interleukin-6 showed a significant positive correlation with total fat on DEXA scan. Linear regression analysis of the adipokines with body-fat distribution parameters revealed that PCOS status had no association between the adipokines and body-fat distribution parameters.
***Conclusion***: PCOS status (i.e., defined as anovulation and polycystic ovaries on ultrasound and/or hyperandrogenaemia) is a not a determining factor in the association between body-fat distribution parameters and serum adipokine levels in women with obesity and infertility.


## INTRODUCTION

Polycystic ovary syndrome (PCOS) affects 5–10% of women of reproductive age and is the most common cause of anovulation. PCOS is associated with a different body-fat distribution and the adipose tissue function and morphology may also be different compared to ovulatory controls (Norman *et al*., 2007). PCOS is defined by two of the

following three criteria: anovulation, polycystic ovary morphology on ultrasound and clinical or biochemical hyperandrogenaemia (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Overweight/obesity and abdominal obesity independently contribute to anovulation in women with PCOS by contributing to insulin resistance (IR), which is considered to play a pivotal role in the pathogenesis of PCOS and anovulation (Pasquali *et al*., 2006; Carmina *et al*., 2007). Abdominal fat can be divided into subcutaneous abdominal fat (SAF) and intra-abdominal fat (IAF). IAF is considered a marker of IR in women with PCOS (Lord *et al*., 2006; Cascella *et al*., 2008) and IAF is associated with IR in premenopausal women even after correcting for SAF (Ross *et al*., 2002). The results of studies assessing the differences between IAF and SAF between women with PCOS and ovulatory controls are inconsistent (Yildirim *et al*., 2003; Barber *et al*., 2008a; Dolfing *et al*., 2011; Manneras-Holm *et al*., 2011) and may be related to the difference in BMI of the study populations. The predictive value of IAF on the risk of IR seems to decrease with increasing BMI as a result of the so-called 'critical intra-abdominal fat threshold' hypothesis (Freedland, 2004). It is postulated that, with increase in body weight, IAF reaches a point of saturation after which fat is shunted to SAF. When the ability of SAF to store excess fat is exhausted, fat is shunted as free fatty acids (FFA) to ectopic sites like the liver, skeletal muscles and pancreas (Weiss, 2007; Koska *et al*., 2008; Stefan *et al*., 2008; Arsenault *et al*., 2011). This accumulation of fat in ectopic sites is strongly associated with IR (Weiss, 2007; Stefan *et al*., 2008). The above-mentioned findings support the notion that with increasing BMI, the measurement of the volumes of IAF and SAF can no longer be used to predict IR due to fat redistribution and accumulation of fat in ectopic sites.

Adipose tissue is not only a site of lipid storage, but a complex endocrine organ expressing and secreting many bioactive peptides, collectively called adipokines. Previous studies suggest that the morphology and function of adipose tissue in PCOS women differs from that of ovulatory controls. In women with PCOS, subcutaneous adipose tissue is characterised by enlarged adipocytes and low-grade inflammation (increased macrophage density), although the latter is not supported by all studies (Escobar-Morreale *et al*., 2011; Lindholm *et al*., 2011; Manneras-Holm *et al*., 2011). In women with PCOS, fat cells of IAF and SAF have different lipolytical activity compared to ovulatory controls (Ek *et al*., 2002; Faulds *et al*., 2003). The hyperlipolytic state of the IAF contributes to drainage of FFA, glycerol and adipokines to the liver contributing to IR.

Adipokines, like leptin, adiponectin, interleukin-6 (IL-6) and tumour necrosis factor α (TNFα) act in an autocrine and paracrine fashion on adipose tissue and in an endocrine manner on distant tissues and organs and they may influence female reproduction by an effect on the hypothalamus, the ovary and the endometrium *(*Mitchell *et al., 2005; Robker et al.,* 2009).

It can be hypothesised that the difference in the body-fat distribution in combination with differences in the morphology and function of the adipose tissue in women with PCOS compared to ovulatory controls could lead to a difference in the secretion of adipokines (Bohler *et al*., 2010). However, leptin levels in obese women with PCOS do not differ significantly from those of age- and weight-matched ovulatory controls (Remsberg *et al*., 2002; Sepilian *et al*., 2006; Carmina *et al*., 2009,). The positive correlation between BMI and leptin is similar in PCOS and ovulatory controls (Brzechffa *et al*., 1996). According to a meta-analysis there are no significant differences in the serum levels of IL-6 and TNFα between PCOS and ovulatory controls even after excluding BMI mismatched studies (Escobar-Morreale *et al*., 2011). Initial studies did not consistently show that serum levels of adiponectin are decreased in women with PCOS but a meta-analysis and more recent studies have shown that after controlling for BMI-related effects, adiponectin levels are lower in women with PCOS compared to ovulatory controls (O'Connor *et al*., 2010; Toulis *et al*., 2009 Manneras-Holm *et al*., 2011). The discrepancy of the findings of previous studies could be explained by the fact that initial studies did not correct for differences in BMI and did not measure the high molecular weight (HMW) adiponectin, which is specifically reduced in women with PCOS (O'Connor *et al*., 2010; Wickham *et al*., 2011).
In a study comparing 48 women with PCOS (BMI 27.1±5.6 kg/m$^2$) to 20 BMI (BMI 26.8±3.9 kg/m$^2$) matched ovulatory controls, PCOS women had significantly more abdominal fat on DEXA, no difference in serum leptin levels, but significantly lower adiponectin levels. Serum leptin showed a positive and adiponectin a negative correlation with all parameters of body-fat distribution (Carmina *et al*., 2009). In other study populations, leptin secretion correlates with fat mass and the secretion is greater from SAF than from IAF (Kershaw and Flier, 2004), while adiponectin shows a negative correlation with IAF and SAF (Jain *et al*., 2009). The release of TNFα is not different between IAF and SAF while IL-6 is preferentially released by IAF (Fain *et al*., 2004). Most studies show a BMI-related difference in the secretion of adipokines by IAF and SAF (Fain, 2010; Kershaw and Flier, 2004), but the redistribution of fat from IAF to SAF and ultimately to ectopic fat sites like the liver, skeletal muscles and pancreas were not measured in these studies. The contribution of IAF and SAF compartments to the serum adipokines in women with anovulatory PCOS needs further assessment. We investigated in women with obesity and infertility, whether PCOS status (i.e., anovulation and polycystic ovaries on ultrasound and/or hyperandrogenaemia) determines the association between the body-fat distribution parameters and the serum adipokine levels.

## MATERIALS AND METHODS

### *Subjects*

All participants were recruited from women with obesity and infertility attending the Fertility clinic of the University Medical Center Groningen (UMCG), The Netherlands with the aim to follow a 6-month lifestyle programme. For the present analysis the participants were selected from a total cohort of 57 participants, published previously as a comparison of body-fat distribution characteristics between ovulatory and anovulatory infertile women with obesity as well as the contribution of IAF and SAF to anovulation *(Kuchenbecker et al.,* 2010). Thirty two anovulatory women with PCOS and 15 ovulatory non-PCOS controls were included in the present analysis. After the fertility work-up, the 15 ovulatory non-PCOS controls were diagnosed as unexplained or mild male factor infertility, whilst anovulation was the cause of infertility in the 32 PCOS women. Participants were diagnosed as PCOS according to the Rotterdam consensus diagnostic criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004) if two of the following criteria were identified: anovulation (in all 32 women), hyperandrogenemia (clinically or biochemically) (in 30 women) or polycystic ovary morphology on ultrasound [≥12 follicles 2–9 mm and/or increased ovarian volume (>10 ml) in at least one ovary] (in 19 women). Hyperandrogenaemia was defined by a serum testosterone level >3.5 nmol/l or a free testosterone level >62 pmol/l or when clinical hirsutism was present. PCOS is a heterogeneous condition and we therefore focussed on the specific subgroup of anovulatory PCOS because of its association with obesity. Of the 15 ovulatory non-PCOS controls, 13 did not show PCO morphology on ultrasound and had no clinical or biochemical hyperandrogenaemia, whilst two women had PCO morphology on ultrasound without clinical of biochemical hyperandrogenaemia. No study subject received hormonal preparations or metformin in the 3 months preceding inclusion. All women were Caucasian, except for one women of Asian origin. The study was approved by the Medical Ethical Committee of the UMCG and informed written consent was obtained from all women.

### *Anthropometric assessment*

The following anthropometric measurements were determined at intake: body weight (up to nearest 0.1 kg), BMI, waist circumference (Wc) (up to the nearest 1 cm). A whole body scan by DEXA assessed the total and trunk fat mass as well as the abdominal fat mass. All participants underwent a single-sliced abdominal CT scan (ssCT) at the level of the umbilicus for the measurement of the IAF and the SAF. A detailed description of the protocol of anthropometric assessments in this study population was published previously (Kuchenbecker *et al*., 2010). Pregnancy was excluded before ssCT by an urinairy pregnancy test.

Abdominal ultrasound (US) was performed using a ALOKA SSD-1000 (Aloka, Tokyo, Japan) ultrasound machine with a 3.5 MHz convex-array abdominal transducer to measure liver-fat accumulation according to a qualitative scoring method (Joseph *et al*., 1991, Mendler *et al*., 1998). Liver-fat accumulation was categorised in four groups: severe, moderate, mild and no liver-fat accumulation. All US measurements were performed by a single observer.

***Biochemical assessment***

All blood samples were taken after an overnight fast of 10 hours. The following blood tests were performed on all samples: total testosterone, sex hormone-binding globulin (SHBG), insulin, glucose, leptin, adiponectin, TNF-α and IL-6. A detailed description of the measurement techniques of total testosterone, SHBG, insulin and glucose in this study population was published previously (Kuchenbecker *et al*., 2010). Homeostasis model assessment score for insulin resistance (HOMA-IR), was calculated as [fasting serum insulin (mU/l) x fasting plasma glucose (mmol/l)] / 22.5 by using the digital HOMA calculator which corrects for insulin measurement techniques (Manley *et al*., 2008; Muniyappa *et al*., 2008). Free testosterone was calculated with the formula according to Vermeulen (Vermeulen *et al*., 1999). In the UMCG, a free testosterone level >62 pmol/l is defined as hyperandrogenemia. Serum levels of leptin were quantified by Enzyme-linked immunosorbent assay (ELISA), (Linco Reasearch, St. Charles, MN, USA) and showed a limit of sensitivity of 0.135 ng/ml with an intra-assay variation of 2.6–4.6% and an inter-assay variation of 2.6–6.2%. The serum adiponectin was also measured by ELISA (Millipore, St. Charles, MN, USA), showing a limit of sensitivity of 0.78 ng/ml with an intra-assay variation of 0.9–7.4% and an inter assay variation of 2.4–8.4%. TNFα and IL-6 were measured by a quantitative sandwich enzyme immunoassay technique (Quantikine HS Immunoassay kit, R&D Systems, McKinley Place, MN, USA) with a limit of sensitivity for TNFα of 0.106 pg/ml with an intra-assay variation of 3.1–8.5% and an inter-assay variation of 7.3–10.6%. Measurement of IL-6 showed a limit of sensitivity of 0.039 pg/ml with an intra-assay variation of 6.9–7.8% and an inter-assay variation of 6.5–9.6%.

***Statistical analysis***

For comparison between groups, subjects were stratified by PCOS status into an anovulatory PCOS group and an ovulatory non-PCOS control group. Inter-group comparisons were made by using a $\chi^2$ test for ordinal variables and an unpaired Student's *t*-test for normally distributed continuous variables or a Mann-Whitney *U*-test when the distribution was skewed. Normal distribution was tested using the Kolmogorov-Smirnov-test. The relationship between body-fat distribution parameters and adipokine levels in the total group of participants were examined using bivariate correlation (Pearson). Linear

regression analysis was performed with the adipokines as dependant variable and the body-fat distribution parameters as covariates correcting for PCOS and in a separate analysis correcting for BMI and age. All analyses were performed using SPSS, version 18 (SPSS Inc., Chicago, IL, USA). Differences or effects were considered statistically significant if $P<0.05$.

## RESULTS

In the total group of 47 women with obesity and infertility, the BMI ranged from 29.2–47.4 kg/m$^2$ and the age ranged from 20–38 years. There was no significant difference in BMI and age between the 32 anovulatory PCOS women and the 15 ovulatory non-PCOS controls (Table 1).

**Table 1**. Characteristics of obese women with infertility comparing women with anovulatory PCOS to ovulatory non-PCOS controls

| Parameter | Total (n = 47) | PCOS (n = 32) | Controls (n = 15) | P |
|---|---|---|---|---|
| Age (years)[a] | 29.2±4.0 | 28.4±3.8 | 30.8±4.2 | 0.061 |
| BMI (kg/m$^2$)[a] | 36.8±4.9 | 37.5±4.9 | 35.2±4.7 | 0.150 |
| Waist circumference (cm)[a] | 110±12 | 113±12 | 104±9 | 0.014* |
| Total fat DEXA (kg)[a] | 44.7±10.3 | 46.1±10.1 | 41.9±10.4 | 0.194 |
| Trunk fat DEXA (kg)[a] | 21.8±5.5 | 23.1±5.5 | 18.9±4.4 | 0.013* |
| Abdomen fat DEXA (kg)[a] | 4.1±1.3 | 4.5±1.3 | 3.4±0.9 | 0.008* |
| IAF ssCT (cm$^3$)[a] | 194±62 | 196±57 | 188±74 | 0.692 |
| SAF ssCT (cm$^3$)[a] | 951±190 | 992±196 | 864±148 | 0.030* |
| Testosterone (nmol/l)[b] | 3.7 (1.6–6.5) | 4.0 (1.6–6.5) | 3.1 (1.6–5.4) | 0.034* |
| SHBGb | 26 (8–93) | 21 (8–43) | 37 (9–93) | 0.007* |
| Fasting insulin (pmol/l)[b] | 146.2 (25.8–400.0) | 164.5 (25.8–400.0) | 125.9 (43.8–301.1) | 0.045* |
| HOMA-IR[b] | 2.6 (0.5–6.2) | 2.8 (0.5–6.2) | 2.1 (0.8–4.0) | 0.081 |
| Leptin (ng/ml)[a] | 47.2±14.4 | 48.5±14.1 | 43.7±15.8 | 0.276 |
| Adiponectin (ng/ml)[b] | 6.4 (1.7–12.0) | 6.4 (3.4–12.0) | 6.7 (1.7–11.1) | 0.603 |
| IL-6 (pg/ml)[b] | 2.4 (0.6–6.2) | 2.4 (0.6–6.2) | 2.4 (0.7–5.5) | 0.423 |
| TNF-α (pg/ml)[b] | 2.2 (0.6–3.9) | 2.4 (0.6–3.8) | 1.9 (0.6–3.9) | 0.133 |

Data expressed as [a] mean±SD using an independent sample Student's *t*-test or as [b] median, minimum and maximum using a Mann-Whitney *U*-test.
[*] Significant at level $P\leq0.05$.

**Table 2.** Liver-fat accumulation in obese women with infertility comparing women with anovulatory PCOS to ovulatory non-PCOS controls

| Liver-fat accumulation | | PCOS (n = 29) | Controls (n = 12) |
|---|---|---|---|
| None | n | 1 | 2 |
| | % of group | 3.4% | 16.7% |
| Mild | n | 11 | 6 |
| | % of group | 37.9% | 50% |
| Moderate | n | 13 | 4 |
| | % of group | 44.8% | 33.3% |
| Severe | n | 4 | 0 |
| | % of group | 13.8% | 0% |

Differences by $\chi^2$ test; P=0.236.



The difference in anthropometric parameters between the anovulatory PCOS and ovulatory non-PCOS controls are shown in Table 1. Anovulatory PCOS women had a significantly higher Wc, abdominal and trunk fat on DEXA scan, significantly more SAF on ssCT, and a non-significant difference in BMI and total fat mass on DEXA scan compared to the ovulatory non-PCOS controls. The IAF on ssCT was not significantly different between anovulatory PCOS women and ovulatory non-PCOS controls. The present study shows more liver-fat accumulation on US in women with anovulatory PCOS compared to the ovulatory non-PCOS group, with moderate and severe liver-fat accumulation in 58.6% and 33.3% of women in the anovulatory PCOS and ovulatory non-PCOS group, respectively (Table 2). These differences were not statistically significant.

The 32 anovulatory PCOS women had significant higher levels of fasting insulin, testosterone and SHBG compared to the 15 ovulatory non-PCOS controls. The HOMA-IR was higher in women with anovulatory PCOS compared to ovulatory non-PCOS controls, but the difference did not reach significance (P=0.081) (Table 1). There was no significant difference in serum levels of leptin, adiponectin, TNF-$\alpha$ and IL-6, between the anovulatory PCOS women and the ovulatory non-PCOS controls (Table 1).

In the total group of 47 women (anovulatory PCOS and ovulatory non-PCOS controls), leptin showed a significant positive correlation with SAF on ssCT, and with trunk fat, abdominal fat and total fat on DEXA scan (r=0.488–0.681; P<0.001). IL-6 showed a significant positive correlation with total fat on DEXA scan (r=0.314; P=0.043) (Table 3). Linear regression analysis of the adipokines with body-fat distribution parameters revealed that PCOS status had no influence on the association. After correcting for BMI and age, PCOS status still had no influence on the association between the adipokines and body-fat distribution parameters.

**Table 3.** Correlation of body-fat distribution parameters, fasting insulin and adipokines in obese women with infertility (total groups of anovulatory PCOS and ovulatory non-PCOS controls)

| | | Insuline | Leptin | Adiponectin | IL-6 | TNFα |
|---|---|---|---|---|---|---|
| IAF ($cm^3$) | Pearson | 0.170 | 0.094 | -0.082 | 0.027 | 0.071 |
| | P | 0.260 | 0.552 | 0.604 | 0.865 | 0.653 |
| SAF ($cm^3$) | Pearson | -0.067 | 0.523** | 0.209 | 0.262 | 0.061 |
| | P | 0.660 | <0.001 | 0.184 | 0.093 | 0.701 |
| AbdDexa (kg) | Pearson | 0.072 | 0.488* | 0.076 | 0.230 | 0.120 |
| | P | 0.636 | <0.001 | 0.630 | 0.143 | 0.448 |
| TrunkDexa (kg) | Pearson | 0.195 | 0.535** | 0.019 | 0.265 | 0.088 |
| | P | 0.195 | <0.001 | 0.905 | 0.090 | 0.578 |
| TotFat (kg) | Pearson | 0.001 | 0.681** | 0.161 | 0.314* | -0.029 |
| | P | 0.997 | <0.001 | 0.307 | 0.043 | 0.855 |

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

## DISCUSSION

This study shows that, in spite of significant differences in body-fat distribution parameters between anovulatory PCOS women and ovulatory non-PCOS controls, PCOS status (i.e., defined as anovulation and polycystic ovaries on ultrasound and/or hyperandrogenaemia) is not a determining factor in the association between serum adipokine levels and body-fat distribution parameters in the total group of women with obesity (mean BMI 36.8±4.9 $kg/m^2$) and infertility.

In accordance with previous studies, the present study confirms that women with anovulatory PCOS who are overweight and obese have abdominal obesity compared to ovulatory non-PCOS controls (Carmina *et al*., 2007). Furthermore, the present study shows that obese women with anovulatory PCOS have significantly more SAF and no difference in IAF compared to obese ovulatory non-PCOS controls. The results of previous studies evaluating the differences in IAF and SAF between women with PCOS and ovulatory controls were inconsistent (Yildirim *et al*., 2003; Barber *et al*., 2008a; Cascella *et al*., 2008; Dolfing *et al*., 2011; Hutchison *et al*., 2011). These study populations had different mean BMI levels and the inclusion criteria for PCOS and the control groups were different between the studies. Two studies used US to measure IAF in stead of the gold standard of Abdominal Computerised Tomography scan or Magnetic Resonance Imaging *(*Yildirim *et al*., 2003; Cascella *et al*., 2008), whilst US measurement of IAF was not validated in women of reproductive age.

The correlation of the body-fat distribution parameters and serum leptin, IL-6 and TNFα levels in women with obesity and infertility (total group) in the present study are in accordance with previous reports (Kershaw and Flier, 2004; Carmina *et al*., 2009; Jain *et al*., 2009). Except for a non-significant negative correlation of adiponectin with IAF, this study did not show a negative correlation of adiponectin with other body-fat distribution parameters. The present study could not confirm the finding of a meta-analysis showing a decrease in serum adiponectin levels in women with PCOS compared to ovulatory controls (Toulis *et al*., 2009). However, more recent studies correcting for differences in BMI and fat mass do not consistently show lower adiponectin and lower high molecular weight (HMW) adiponectin levels in women with PCOS compared to ovulatory controls *(Barber et al.*, 2008b; O'Connor *et al*., 2010; Wickham *et al*., 2011). Adiponectin levels follow a circadian pattern and can be influenced by high-carbohydrate meals, dietary supplements and oxidative stress (Bohler *et al*., 2010), factors which have not been reported in the abovementioned meta-analysis. Future studies on adiponectin levels should attempt to eliminate and correct for these influences and should attempt to measure the HMW adiponectin because it most closely correlates with the measures of insulin sensitivity (Fisher *et al*., 2005; Nakashima *et al*., 2006).

In spite of a significant increase in abdominal obesity measures and SAF and the known difference in the morphology and function of adipose tissue in obese women with anovulatory PCOS compared to obese anovulatory non-PCOS controls, linear regression analysis correcting for BMI and age, did not reveal that PCOS status is a determining factor in the correlation between body-fat distribution and serum adipokine levels. Two possible explanations for this finding should be considered.

The first explanation is based on the process of body-fat redistribution as described in the introduction. During chronic exposure to energy dens diets, subcutaneous adipose tissue needs to regulate the recruitment, differentiation and proliferation of preadipocytes to mature adipocytes (adipogenesis), leading to more adipocytes and an increased storage capacity of excess fat (hyperplastic obesity) (Danforth, 2000; Rodriguez-Acebes *et al*., 2010). In many obese patients adipogenesis may become dysregulated, resulting in dysfunctional subcutaneous adipose tissue in many obese patients, leading to adipocyte hypertrophy and decreased fat-storage capacity (hypertrophic obesity) (Danforth, 2000; Villa and Pratley, 2011). Hypertrophic obesity is associated with increased fat cell size, necrosis and an inflammatory response (Weiss, 2007; Rasouli and Kern, 2008), resulting in a decreased ability to store additional fat and the secretion of a different adipokine profile. Dysfunctional subcutaneous adipose tissue contributes to the process of body-fat redistribution with shunting of excess fat from subcutaneous adipose tissue to ectopic sites like the liver, skeletal muscles and pancreas. Accumulation of fat in these ectopic sites is strongly associated with IR and an adverse metabolic profile (Weiss, 2007; Koska *et al*.,

2008; Arsenault *et al*., 2011). Individuals with hyperplastic obesity on the other hand can store increased amount of fat in the subcutaneous adipose tissue without shunting of fat to ectopic sites. Individuals with hyperplastic obesity have limited IR and a favourable metabolic profile and are described as having a metabolically healthy obese phenotype (Stefan *et al*., 2008; Hayes *et al*., 2010). In Figure 1 adipose tissue dysfunction and the consequences of body-fat redistribution is schematically presented.

We hypothesize that in women with infertility and high BMI levels, adipose tissue dysfunction occurs resulting in body-fat redistribution with fat shunted from IAF to SAF and later to ectopic sites. This explains the findings of the present study that in women with anovulatory PCOS and obesity, SAF and not IAF volume is increased, as well as the non-significant increase of moderate and severe liver-fat accumulation in anovulatory PCOS women compared to ovulatory non-PCOS controls. In anovulatory PCOS women with lower BMI levels and no adipose tissue dysfunction, body-fat distribution parameters may be different compared to women with higher BMI levels, because body-fat redistribution may not have been initiated in the anovulatory PCOS women with low BMI.

Recent data indicate that the adipogenesis in PCOS women may be altered due to decreased expression of genes involved in the proliferation of omental fat (Corton *et al*., 2007). Some studies indicate that androgen excess in women with PCOS may also contribute to impaired adipogenesis. Dihydrotestosterone decreases the differentiation and lipid accumulation in human preadipocytes and adipocytes in culture (Gupta *et al*., 2008). Prenatal exposure to androgens in animal models induces increased adipocyte cell size accompanied by IR (Roland *et al*., 2010). It might be hypothesised, that androgen excess in women with PCOS may initiate the process of adipose tissue dysfunction at a lower BMI level compared to non-PCOS controls.

The second explanation of the main finding of the present study is that adipokine serum levels may not accurately reflect the function of adipose tissue compartments. Some adipokines are exclusively secreted by adipocytes (like adiponectin) while others (like leptin) are also expressed by other tissues (Rasouli and Kern, 2008; Bohler *et al*., 2010). Only one-third of circulating IL-6 originates from adipocytes and like TNFα, it acts mostly in an autocrine and paracrine manner (Bohler *et al*., 2010). The release of adipokines and FFA by IAF into the portal venous system to the liver contributes to significant metabolic changes, which will not accurately be reflected by their serum levels (Weiss, 2007). Furthermore, the contribution of ectopic fat depots, like the liver, skeletal muscle and pancreas, to serum adipokine levels needs further assessment in women with PCOS.

The following limitations of our study should be considered. Due to the limited number of participants, a sub-analysis of different BMI groups was not possible and subtle differences in the serum adipokine levels and findings of the correlations between body-fat distribution parameters and adipokines may have been missed.

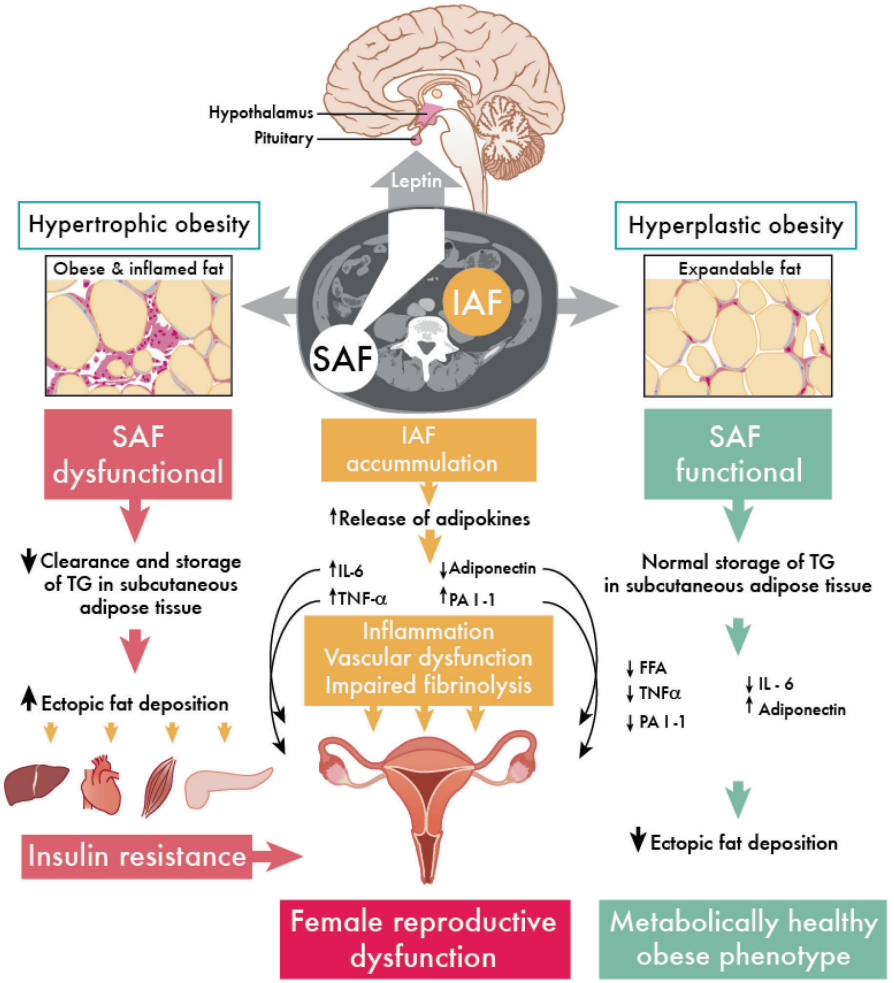


**Figure 1.** Proposed mechanisms by which accumulation of intra-abdominal (IAF) and sub-cutaneous abdominal fat (SAF) can lead to dysfunctional and functional adipose tissue, and be linked to metabolic and female reproductive consequences of obesity.

FFA, free fatty acids
IL-6, interleukin-6
PAI-1, plasminogen activator inhibitor-1
TG, triglycerides
TNFα, tumour necrosis factor α

Another limitation of our study is that IAF and SAF were measured by ssCT scan at the level of L4-L5, and that the outcome depends on the individual variation of IAF and SAF distribution. A study published after initiation of ours, indicates that in women the measurement of IAF by single-sliced CT scan at the level of L2-L3 shows better correlation with whole abdomen CT scan (Kuk *et al*., 2010). In the present study we did not measure the HMW adiponectin levels, which might give a better reflection of the level of IR.

In conclusion, in women with obesity and infertility and a mean BMI of 36.8±4.9 kg/m$^2$, PCOS (i.e., defined as anovulation and polycystic ovaries on ultrasound and/or hyper-androgenaemia) is not a determining factor in the association between the body-fat distribution parameters and serum adipokine levels. The explanation of the discrepancy of our results with previous studies in women with PCOS and lower BMI levels may be that with high BMI levels, adipose tissue dysfunction occurs leading to body-fat redistribution and altered adipokine secretion. With increasing BMI, the differences in body-fat distribution between women with anovulatory PCOS and ovulatory non-PCOS controls are therefore overruled by body-fat redistribution. Future studies should aim to analyse adipose tissue (including omental fat and ectopic fat mass) features in women with PCOS over a wide range of BMI levels, in order to evaluate the difference in adipogenesis and the effect of adipose tissue dysfunction on the metabolic en reproductive consequences of obesity.

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Chapter 6

# In women with polycystic ovary syndrome and obesity, loss of intra-abdominal fat is associated with resumption of ovulation

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## ABSTRACT

***Background*:** It is not clear why some anovulatory women with polycystic ovary syndrome (PCOS) and obesity resume ovulation and others remain anovulatory after weight loss. The objective of this study was to compare the changes in body-fat distribution and specifically intra-abdominal fat (IAF) and subcutaneous abdominal fat (SAF) between a group of anovulatory women with PCOS and obesity who resume ovulation (RO+) to those who remain anovulatory (RO-) during a lifestyle programme.

***Methods***: In a prospective pilot cohort study, anovulatory women with PCOS underwent a 6-month lifestyle programme in a tertiary fertility clinic. Body-fat distribution was assessed by anthropometrics, dual-energy x-ray absorptiometry (DEXA), single-slice abdominal CT scan (ssCT) at intake, after 3 months and after 6 months. Baseline-corrected changes over time were analysed using Generalised Estimating Equations (GEE) longitudinal regression analysis.

***Results***: In 32 anovulatory women with PCOS (age 28±4 years; BMI 37.5±5.0 kg/m²), there were no significant baseline differences in anthropometrics and biochemical assessment between 14 RO+ participants and 18 RO- participants. RO+ women lost more weight (6.3% versus 3.0%) and abdominal fat on DEXA (15.0% versus 4.3%) compared to RO- women. Resumption of ovulation was associated with early and consistent loss of IAF (12.4% versus 5.0% at 3 months and 18.5% versus 8.6% at 6 months). Loss of SAF between the RO+ women and the RO- women was similar at 3 months (6.2% versus 6.1%) but did not change any further in RO- women (6.1 %) as it did in RO+ women (11.4 %) at 6 months.

***Conclusions***: In anovulatory women with PCOS and obesity undergoing a lifestyle programme, RO+ women lose more body weight and abdominal fat on DEXA than RO- women. In addition, this study shows that early and consistent loss of IAF is associated with resumption of ovulation. Future studies should address the mechanisms behind these changes and should assess interventions aimed at loss of IAF to facilitate resumption of ovulation.




## INTRODUCTION

The high prevalence of overweight (body mass index (BMI) 25–29.9 kg/m$^2$) and obesity (BMI ≥30 kg/m$^2$) is significantly contributing to the overall burden of disease worldwide, and the effect on female reproduction is a growing additional concern (Haslam and James, 2005; Nelson and Fleming, 2007). Overweight and obesity in women is a main contributor to anovulation, with an exponential increase in anovulation with increasing body weight (Green *et al*., 1988; Rich-Edwards *et al*., 1994).

Increased abdominal fat accumulation (waist hip ratio >0.8 in women) contributes to reproductive dysfunction (Zaadstra *et al*., 1993; Wass *et al*., 1997; Pasquali *et al.*, 2006) and these findings are unchanged after correcting for BMI. Abdominal fat accumulation is an indicator of higher metabolic risk profile because it is associated with insulin resistance (IR) (Despres *et al*., 2001). Hyperinsulinaemia in women with obesity contributes to anovulation by increased ovarian androgen secretion (Pasquali *et al*., 2006; Bohler *et al.*, 2010), leading to arrest of follicle growth (Willis *et al*., 1996).

Loss of abdominal fat, on the other hand, is associated with resumption of menstruation and ovulation in obese women with polycystic ovary syndrome (PCOS) undergoing a weight loss programme (Huber-Buchholz *et al*., 1999; Thomson *et al.*, 2008). It has been shown that resumption of ovulation in anovulatory women with obesity undergoing weight loss is mediated by improvement of IR and decrease in free androgen levels (Guzick *et al*., 1994; Holte *et al*., 1995; Pasquali *et al*., 2006). Abdominal fat consists of intra-abdominal fat (IAF) and subcutaneous abdominal fat (SAF). In study subjects with obesity who lost weight, loss of IAF had a greater beneficial effect on IR than loss of SAF (Park and Lee 2005). The independent contribution of exercise to the improvement of IR and loss of IAF is not clear because only few studies tried to correct for diet and weight loss (Carroll and Dudfield, 2004; Christiansen *et al*., 2009; Hutchison *et al*., 2011).

It remains unexplained why some women remain anovulatory and others resume ovulation after weight loss. It can be hypothesised that specifically loss of IAF is required for improvement of IR, decease in androgen levels and resumption of ovulation. We tested this hypothesis by comparing the changes in body-fat distribution, and especially IAF and SAF, in a group of obese anovulatory women with PCOS who resumed ovulation to those who remained anovulatory during a 6-month lifestyle programme.

## MATERIALS AND METHODS

### *Population*

In a pilot prospective cohort study, participants were recruited from women with obesity and infertility attending the Fertility Clinic of the University Medical Center Groningen (UMCG) between 2005 and 2008. All women with a BMI >29 kg/m$^2$ who met the inclusion criteria (infertility $\geq$1 year, age <38 years, partner with total motile sperm concentration/ejaculate $\geq$10 million) were asked to participate in a 6-month lifestyle programme. The baseline data of this cohort of women before start of the lifestyle programme were published previously as a comparison of body-fat distribution characteristics between ovulatory and anovulatory infertile women (Kuchenbecker *et al*.,

2010). In the present study, assessing changes during the lifestyle programme, only anovulatory women with PCOS were included in the analysis.
Participants were diagnosed as PCOS according to the Rotterdam consensus diagnostic criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004) if two of the following criteria were identified: anovulation, hyperandrogenaemia (clinically or biochemically) or polycystic ovary morphology on ultrasound and after other endocrine causes of anovulation were excluded. Hyperandrogenaemia was defined by a serum testosterone level >3.5 nmol/l or a free testosterone level >62 pmol/l or when clinical hirsutism was present. No participant received hormonal medications or metformin in the 3 months preceding inclusion. All participants were Caucasian, except for one participant of Asian origin.

Women with amenorrhea (cycle interval ≥6 months) were considered anovulatory. Women with cycles ≤42 days in whom ovulation could not be confirmed by ultrasound monitoring, as well as women with menstrual cycles between 42 days and 6 months kept a basal body temperature (BBT) chart. Women with no BBT rise or a midluteal progesterone level ≤15 nmol/l, 1 week after a BBT rise, were considered anovulatory. In all study subjects, hyperprolactinaemia, abnormal thyroid function, non-classical 21-hydroxylase deficiency and an androgen secreting tumour was excluded.
During the lifestyle programme, all women kept a BBT chart and a midluteal progesterone level was assessed 1 week after a maintained temperature rise. Resumption of ovulation was defined as a midluteal progesterone level >15 nmol/l at least once, or by the occurrence of conception.
Participants who did not resume ovulation (RO-) and those who resumed ovulation (RO+) but did not conceive remained in the 6-month lifestyle programme. Women who conceived did not continue the lifestyle programme. Women who stopped the lifestyle programme before the 6th month were considered to be drop-outs. All drop-outs were contacted by telephone 6 months after drop-out to inquire about self-reported weight loss and conception.
The study was approved by the Medical Ethical Committee of the UMCG and informed written consent was obtained from all subjects.
The lifestyle programme of 6 months was based on the National Institute of Health evidence report of the treatment of obesity (National Heart, Lung and Blood Institute/National Institutes of Diabetes and Digestive and Kidney diseases, 1998) and consisted of a combination of dietary advice, increased physical activity and behaviour modification.

***Anthropometric assessment***

Body weight (kg), height (cm), BMI (weight (kg) divided by height in meters$^2$), waist circumference (Wc, waist measured at the narrowest part of the torso located between the lower rib and the iliac crest), a whole body DEXA scan (DEXA) and a single-slice abdominal CT scan (ssCT) were measured at baseline. All measurements were carried out by the same observer. Bodyweight was recorded every 2 weeks and the Wc, DEXA and the ssCT were measured again at month 3 and month 6 of the lifestyle programme.

Total, trunk and abdominal fat mass was measured by DEXA, using a Hologic A Discovery Bone Densitometer (Hologic Inc., Bedford, MA, USA). Total fat mass was determined for the whole body, and by using computer programming, two regions (trunk and abdominal slice) were specified and measured as mentioned in previous studies (Carmina *et al*., 2007).

The ssCT consisting of three to four subslices was performed at the level of the umbilicus for the measurement of the IAF and the SAF (Seidell *et al*., 1990). The technique used to measure IAF and SAF was published previously (Kuchenbecker *et al*., 2010). In short, the IAF and SAF volume (cm$^3$) was calculated by multiplying the IAF and SAF area (cm$^2$) of each subslice with the subslice thickness (cm), and the mean volume (cm$^3$) of the subslices was recorded for analysis. All measurements were performed by a single observer.

Pregnancy was excluded before each DEXA and ssCT scan by a urine pregnancy test.

***Biochemical assessment***

Total testosterone, sex hormone binding globulin (SHBG) and insulin were measured after an overnight fast of 10 hours at intake, after 3 months and after 6 months of the lifestyle programme. The formula according to Vermeulen (Vermeulen *et al*., 1999) was used to calculate free testosterone. The exact measurement techniques used for the biochemical assessments were published previously (Kuchenbecker *et al*., 2010).

***Lifestyle programme***

All participants received individualised dietary advice aiming for reduction in calorie intake of at least 500 kcal/day, but avoiding a total calorie intake <1200 kcal/day. An individualised exercise programme was tailored to the ability and personal and social circumstances of each participant. Each participant kept a diet record and carried a pedometer SW-200 (New Lifestyles, Lee's Summit, MO, USA) to record the amount of steps over 2-week periods during the 6-month programme. Individual guidance by a nurse practitioner consisted of visits every 2 weeks, during which body weight was measured and compliance was assessed by evaluating the diet and pedometer records completed at home. In addition, the BBT chart was evaluated and a serum midluteal progesterone level assessment was scheduled if appropriate. By means of motivational counselling techniques,

problems of and resistance to lifestyle changes and behaviour modification were addressed and advice was given (Levensky *et al*., 2007).

***Statistical analysis***

At the end of the lifestyle programme, the participants were divided into those who had resumed ovulation (RO+) and those who had not resumed ovulation (RO-) and post-hoc comparison was performed between the two groups at baseline. The drop-outs were contacted by telephone 6 months after drop-out. If no further weight loss or conception after drop-out was reported, the drop-outs were analysed as part of the RO- group until the time of drop-out.

Between-group comparisons were performed using an independent samples Student's *t*-test for normally distributed continuous variables or a Mann-Whitney *U*-test when the distribution was skewed. Normal distribution was tested using the Kolmogorov-Smirnov-test. Changes over time were analysed using Generalised Estimating Equations (GEE) longitudinal regression analysis with time, ovulatory status, and an interaction term of ovulatory status and time as the predictors. GEE makes maximum use of the available measurements in a longitudinal design without the limitations of complete case analysis. For interventions such as this lifestyle programme, complete case analysis does not reflect a real-life scenario and poses the risk of negative selection since only those who do not conceive or resume ovulation remain in the programme. Baseline correction was performed by including the measurement at T=0 of the respective parameter as a covariate in the GEE analysis. Participants remained in the GEE analysis until conception or drop-out. For this pilot study, no sample-size calculation could be performed. All analyses were performed using SPSS, versions 16 and 17 (SPSS Inc., Chicago, IL, USA). Differences or effects were considered statistically significant if $P<0.05$.

## RESULTS

***Clinical characteristics of participants***

Thirty two anovulatory women with PCOS and obesity were included in the lifestyle programme and their baseline data were recorded. During the first 3 months, the four women that conceived and four drop-outs left the programme, while another five women who resumed ovulation continued the lifestyle programme, therefore leaving 24 participants to measure and record the data at 3 months. Out of the 24 participants continuing the second 3 months of the lifestyle programme, the three women who conceived and six drop-outs left the programme, leaving 15 participants to measure and record the data at 6 months.

**Table 1**. Baseline characteristics of anovulatory women with PCOS and obesity undergoing a 6-month lifestyle programme: comparison of the 10 drop-outs with the participants who completed the lifestyle programme

| Parameter | Completed (n = 22) | Drop-out (n = 10) | P value |
|---|---|---|---|
| Age (years)[a] | 28.9 ± 4.1 | 27.5 ± 2.9 | 0.35 |
| BMI (kg/m$^2$)[a] | 37.8 ± 5.2 | 36.7 ± 4.3 | 0.56 |
| Waist circumference (cm)[a] | 114 ± 13 | 110 ± 9 | 0.36 |
| Total fat DEXA (kg)[a] | 47.6 ± 10.1 | 42.7 ± 9.7 | 0.21 |
| Trunk fat DEXA (kg)[a] | 23.8 ± 5.8 | 21.7 ± 4.9 | 0.34 |
| Abdomen fat DEXA (kg)[a] | 4.7 ± 1.3 | 3.9 ± 1.3 | 0.14 |
| IAF ssCT (cm3)[a] | 197 ± 61 | 195 ± 51 | 0.96 |
| SAF ssCT (cm3)[a] | 1023 ± 183 | 971 ± 223 | 0.50 |
| Testosterone (pmol/l)[b] | 3.7 (1.6 – 6.1) | 4.7 (1.6 – 6.5) | 0.04[*] |
| SHBG (nmol/l)[b] | 22 (10 – 38) | 20 (8 – 36) | 0.72 |
| Free testosterone (pmol/l)[b] | 87 (32 – 169) | 116 (32 – 217) | 0.04[*] |
| Fasting insulin (pmol/l)[b] | 170.3 (25.8 – 602.7) | 193.0 (25.8 – 595.2) | 0.80 |

Data expressed as [a] mean ±SD using an independent sample Student's *t*-test or as [b] median, minimum and maximum using a Mann-Whitney *U*-test.
[*] Significant at level P≤0.05.

None of the 10 drop-outs reported any further weight loss or conception until 6 months after drop-out. They were therefore analysed as part of the RO- group. To ensure that the anthropometric and biochemical measurements of the drop-outs did not differ from the participants that completed the study, we performed a comparison of the baseline characteristics between both groups (Table 1). There was no difference in age, BMI and anthropometric assessment between the drop-outs and the participants that completed the study. Except for significantly higher free testosterone and total testosterone level in the drop-outs (P=0.04), there were no baseline differences in the biochemical assessments between the drop-outs and the participants who completed the study.
The baseline data of the RO+ (n=14) and RO- (n=18) women are shown in Table 2. There was no difference in age, BMI, anthropometric- and biochemical assessment at baseline between the RO+ and RO- women.

***Changes during the lifestyle programme***
At the end of the lifestyle programme, the participants were divided into those who had resumed ovulation (RO+) and those who had not resumed ovulation (RO-) and GEE analysis was performed on the two groups. The unadjusted results of the measurements of the two groups at baseline, month 3 and month 6 are presented in Table 2.

**Table 2**. Characteristics of 32 anovulatory women with PCOS and obesity undergoing a lifestyle programme divided into those who resumed ovulation (RO+) and those who remained anovulatory (RO-) at the end of 6 months. Comparison of baseline characteristics and characteristics of RO+ and RO- women at month 3 and month 6 are given.

| | Baseline | | | Month 3 | | Month 6 | |
|---|---|---|---|---|---|---|---|
| | RO+ n = 14 | RO- n = 18 | P value | RO+ n = 10 | RO- n = 14 | RO+ n = 7 | RO- n = 8 |
| Age (years) [a] | 29.6±4.0 | 27.5±3.4 | 0.11 | | | | |
| BMI (kg/m$^2$) [a] | 37.7±4.5 | 37.3±5.4 | 0.83 | 35.4±4.8 | 37.3±5.7 | 32.2±4.5 | 36.1±7.4 |
| Waist circumference (cm) [a] | 114±13 | 112±11 | 0.59 | 108±15 | 112±13 | 103±8 | 112±19 |
| Total fat DEXA (kg) [a] | 47.7±8.8 | 44.8±11.0 | 0.43 | 43.9±9.9 | 45.9±10.2 | 38.5±7.8 | 44.6±13.1 |
| Trunk fat DEXA (kg) [a] | 23.4±5.2 | 22.9±5.8 | 0.83 | 21.2±5.3 | 22.8±5.2 | 18.4±3.9 | 22.7±6.7 |
| Abdomen fat DEXA (kg) [a] | 4.6±1.4 | 4.4±1.4 | 0.58 | 4.2±1.5 | 4.5±1.4 | 3.6±1.3 | 4.6±1.5 |
| IAF ssCT (cm$^3$) [a] | 191±41 | 201±69 | 0.63 | 156±26 | 206±70 | 133±19 | 192±94 |
| SAF ssCT (cm$^3$) [a] | 1024±187 | 991±205 | 0.65 | 924±215 | 971±170 | 847±235 | 957±241 |
| Total testosterone (pmol/l) [b] | 3.6 (1.6–5.5) | 4.3 (2.8–6.5) | 0.27 | 2.9 (2.3–3.8) | 3.5 (2.4–6.0) | 3.6 (3.3–3.9) | 3.8 (2.4–5.0) |
| SHBG (nmol/l) [b] | 23 (10–43) | 20 (8–36) | 0.33 | 22 (14–31) | 23 (12–39) | 31 (19–47) | 19 (15–26) |
| Free testosterone (pmol/l) [b] | 84 (32–169) | 107 (69–217) | 0.05 | 68 (48–95) | 80 (49–117) | 71 (54–81) | 95 (58–137) |
| Fasting insulin (pmol/l) [b] | 171 (26–603) | 183 (79–596) | 0.56 | 155 (72–237) | 183 (79–545) | 117 (108–151) | 159 (86–215) |
| Pedometer (steps/day) | 7609±2313 | 6373±2873 | 0.22 | 9633±2505 | 7489±3406 | 10528±2109 | 9024±2417 |

Data expressed as [a] mean±SD were analysed using the independent samples Student's *t*-test; data expressed as [b] median, minimum and maximum were analysed using the Mann-Whitney *U*-test.

The results of the GEE analyses of changes over time in body weight and abdominal fat on DEXA are presented in Figure 1. Since GEE was performed with correction for baseline the values differ from those in Table 2. The total group of RO+ women lost more weight (6.3% versus 3.0% at 6 months, P=0.018, Figure 1a) and abdominal fat on DEXA (15.0% versus 4.3% at 6 months, P=0.025, Figure 1b) compared to the total group of RO- women. Figure 2 shows that the RO+ women lost more IAF on ssCT between baseline and month 3 (12.4% versus 5.0%, P=0.002, Figure 2a) and baseline and month 6 (18.5% versus 8.6%, P=0.005, Figure 2a) compared to the RO- women. For SAF on ssCT, on the other hand, changes for RO+ women and RO- women were similar between baseline and month 3 (6.2% versus 6.1%, P=0.946, Figure 2b). Due to continued loss of SAF after 3 months in RO+ women, there was a difference in loss of SAF between baseline and 6 months (11.4% versus 6.1%, P=0.031, Figure 2b) compared to RO- women.

GEE analysis showed a non-significant decrease of fasting insulin (P=0.194) and free testosterone (P=0.086) in the RO+ women compared to the RO- women. The increase in pedometers steps in RO+ women compared to RO- women did not reach statistical significance.

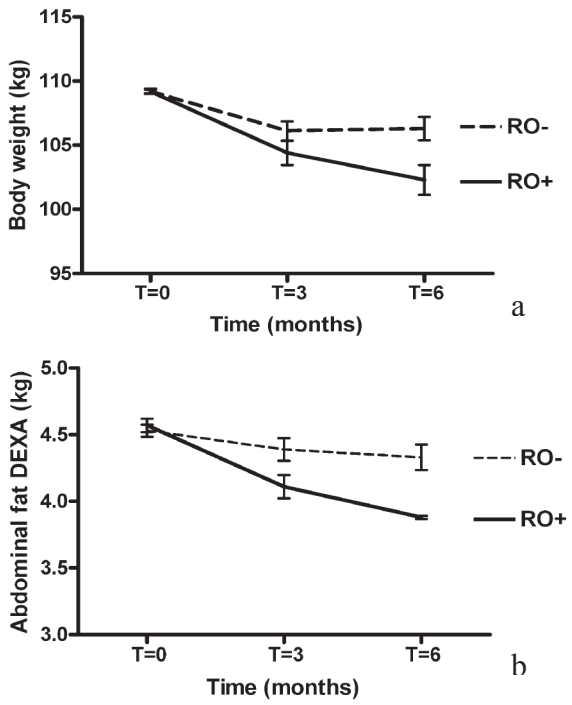


**Figure 1.** Baseline corrected GEE analysis of 32 anovulatory women with PCOS and obesity undergoing a lifestyle program with post hoc allocation of participants into those who resumed ovulation (RO+) and those who remained anovulatory (RO–) at the end of 6 months shows a difference in loss of body weight (P ¼ 0.018) (**a**) and abdominal fat on DEXA (P¼ 0.025) and (**b**) between RO+ (solid line) and RO– (dashed line) participants. Error bars indicate standard errors of GEE estimates.

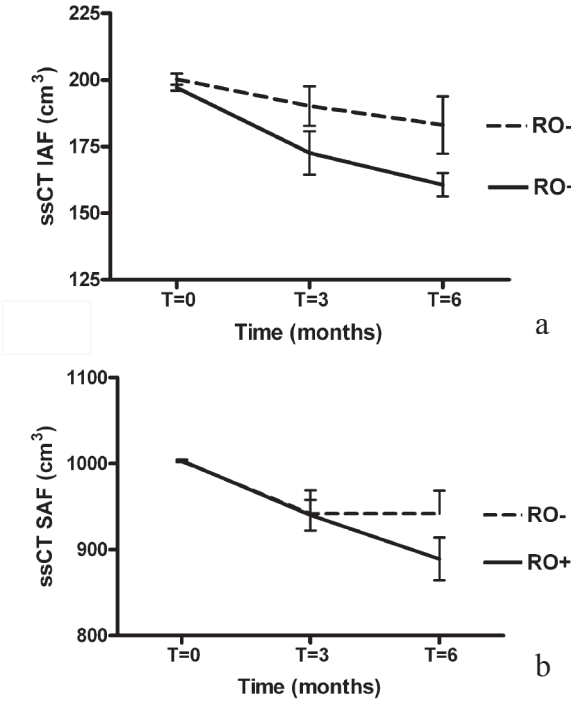


**Fig 2.** Baseline-corrected GEE analysis of 32 anovulatory women with PCOS and obesity undergoing a lifestyle program with post hoc allocation of participants into those who resumed ovulation (RO+) and those who remained anovulatory (RO–) at the end of 6 months show a difference in loss of IAF from month 0 to month 3 (P¼ 0.002) (**a**) and from month 0 to month 6 (P¼ 0.005) (**a**), and difference in loss of SAF from month 0 to month 3 (P¼ 0.946) (**b**) and from month 0 to month 6 (P¼ 0.031) and (**b**) between RO+ (solid line) and RO– (dashed line) participants. Error bars indicate standard errors of GEE estimates.



## DISCUSSION

This study shows that in anovulatory women with PCOS and obesity participating in a lifestyle programme, resumption of ovulation was associated with more weight loss and loss of abdominal fat on DEXA. Resumption of ovulation was associated with early and consistent loss of IAF.

In anovulatory women, >5% loss of body weight is required for resumption of ovulation ( Kiddy *et al*., 1992; Guzick *et al*., 1994; Clark *et al*., 1995; Holte *et al*., 1995; Huber-Buchholz *et al*., 1999). Previous studies using DEXA to quantify the changes in abdominal fat in women with PCOS undergoing a weight loss programme, indicated that loss of abdominal fat is associated with resumption of ovulation (Huber-Buchholz *et al*., 1999; Thomson *et al*., 2008). The present study confirms these findings, since the RO+ women lost 6.3% of their body weight and 15.0% abdominal fat on DEXA during the lifestyle

programme, as compared to 3.0% loss of body weight and 4.3% loss of abdominal fat on DEXA in RO- women. Compared to RO- women, RO+ women lost on average 4.0 kg more body weight and 0.5 kg more abdominal fat on DEXA.

Moreover, our study shows that early and consistent loss of IAF over a 6-month period is associated with resumption of ovulation. Only after more than 3 months of lifestyle intervention, RO+ women started losing more SAF than RO- women. Previous studies in different patient populations have shown that during the initial period of calorie restriction, preferential loss of IAF occurs which correlates significantly with improvement in IR (Goodpaster *et al*., 1999; Park and Lee, 2005). Loss of IAF and not SAF during initial calorie restriction can be explained by increased lipolysis of IAF due to its higher metabolic activity (Smith and Zachwieja, 1999). After surgical removal of IAF, IR has been shown to improve (Thorne *et al*., 2002), while removal of SAF by liposuction does not significantly alter IR (Klein *et al*., 2004). Based on the above-mentioned mechanism, the early and consistent loss of IAF in RO+ women might have contributed to improvement in IR and resumption of ovulation. We could not confirm the expected difference in fasting insulin levels between RO+ and RO- women in our study, which may be due to the high average BMI in our population. Moderate weight loss in women with BMI >35 kg/m$^2$ does not always lead to the same decrease in fasting insulin levels as in women with lower BMI, as shown in a previous study in women with PCOS and obesity (Tang *et al*., 2006).

We have previously shown that in women with obesity and infertility, anovulatory women have significantly more SAF and higher fasting insulin levels and the same amount of IAF compared to ovulatory women with the same BMI (Kuchenbecker *et al*., 2010). A possible explanation for an increased volume of SAF in anovulatory women with obesity could be provided by the concept of a critical IAF threshold. This concept suggests that during constant high calorie food consumption, storage of fat in IAF reaches a point of saturation, after which fat is shunted to the subcutaneous fat compartments (Freedland, 2004). With increased accumulation of SAF, inflammatory changes and increase in adipocyte size occur, limiting the SAF storage capacity and contributing to the antilipolytical effects of insulin. Fat is then shunted from SAF to the liver and skeletal muscle which contributes to increased insulin resistance (Weiss, 2007; Koska *et al*., 2008). IAF, liverfat and skeletal muscle fat due to the higher lipolytical activity of these fat compartments compared to SAF. This mobilization of fat from IAF, liver fat and skeletal muscle fat leads to improved IR (Goodpaster *et al*., 1999; Freedland, 2004). This mechanism could also explain the findings of the present study that early and consistent loss of IAF and not SAF was associated with resumption of ovulation.

Previous studies suggest that exercise contributes to loss of IAF (Kay and Fiatarone Singh, 2006; Ohkawara *et al*., 2007) while another study could not show an independent effect of exercise on loss of IAF (Christiansen *et al*., 2009). In anovulatory women with PCOS, a

structured exercise programme showed higher ovulation rates and improvement in IR compared to a diet programme alone (Palomba *et al*., 2008). A more recent study showed that exercise was associated with loss of IAF and improvement in IR in PCOS women, despite weight maintenance. Using the euglycemic hyperinsulinemic clamp technique, no significant correlation was found; however, between improvement in IR and loss of IAF (Hutchison *et al*., 2011). Exercise might also improve insulin resistance by increasing muscle mass and enhancing glucose disposal in skeletal muscle (Carroll and Dudfield, 2004; Thomson *et al*., 2008). In the present study, we cannot exclude that the non-significant increase in pedometer steps might have contributed to increased loss of IAF and resumption of ovulation in the RO+ women compared to RO- women.

As shown in previous studies, resumption of ovulation during weight loss is associated with a decrease in fasting insulin and free testosterone levels (Guzick *et al*., 1994; Pasquali *et al*., 2006). In addition, weight loss with improvement in IR and therefore lower insulin levels, leads to less androgen production by the ovarian theca cells and more SHBG production in the liver (Poretsky, 1991). Lower free androgen levels in the long term, in turn limits the amount of abdominal fat accumulation (Bohler *et al*., 2010; Pasquali *et al*., 2006). In our study, we found a decrease in free testosterone in the RO+ women that was not statistically significant compared to RO- women. This might be due to small numbers in our study.

This study shows that in anovulatory women with PCOS and obesity, participating in a lifestyle programme, resumption of ovulation is associated with early and consistent loss of IAF. The most likely mechanism of resumption of ovulation after loss of IAF during a lifestyle programme is improvement of IR and lower free androgen levels but other mechanisms should also be considered. Women with anovulatory PCOS have altered adipocytokine secretion (Carmina *et al*., 2009) and changes in the adipocytokine secretion due to loss of IAF can also be considered as a mechanism of resumption of ovulation, as these substances also have direct effects on the ovary (Mitchell *et al*., 2005). Future studies should try to evaluate interventions aimed at loss of IAF and should try to explore the mechanism involved in resumption of ovulation due to loss of IAF.

The present study was a pilot to investigate the feasibility of a lifestyle programme in a tertiary fertility centre. In spite of personal guidance by a nurse practitioner using motivational interviewing techniques, there was a high drop-out rate of 31.3%. Our drop-out rate is in agreement with the rates of 27–35% reported in previous studies on lifestyle intervention in comparable patient populations (Clark *et al*., 1995; Hoeger *et al*., 2004; Palomba *et al*., 2008). Based on follow-up data until 6 months after drop-out, we decided to allocate the drop-outs to the RO- group until the moment of drop-out. This decision was based on the fact that resumption of ovulation is highly unlikely in anovulatory women with obesity who do not lose weight (Clark *et al*., 1995). We can however not exclude the

possibility that women in the drop-out group had occasional ovulation in spite of no further weight loss. The high drop-out rate deserves further evaluation in order to improve the effectiveness of lifestyle programmes. If predictive factors for drop-out in this patient population could be indentified (Teixeira *et al*., 2005), specific personal guidance and intervention should be developed to improve motivation and adherence to diet – and exercise programmes to achieve weight loss.

In conclusion, this study indicates that anovulatory women with PCOS and obesity who resume ovulation during a lifestyle programme lose 4 kg (3.3%) more weight and 0.5 kg (10.7%) more abdominal fat on DEXA than the women who remain anovulatory. Moreover, early and consistent loss of IAF is associated with resumption of ovulation.

Future larger studies on lifestyle interventions in anovulatory women with PCOS should aim to confirm our findings and to evaluate the mechanisms of resumption of ovulation. Trials should be designed to assess the combination of diet and structured exercise programmes aimed at loss of IAF and improvement of IR for resumption of ovulation, thereby decreasing the number of infertile women requiring ovulation induction.

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Chapter 7

# Systematic review: Insulin-sensitizing drugs for weight loss in women of reproductive age who are overweight or obese

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## ABSTRACT

***Background***: Women of reproductive age who are overweight or obese are prone to suffering from infertility. Weight loss in these women leads to increased fecundity, higher chances of conception after infertility treatment and improved pregnancy outcome. In spite of the advantageous effects of weight loss, most patients have difficulty losing weight and often regain lost weight over time. The objective of this review was to assess whether treatment with insulin-sensitizing drugs contributes to weight loss, compared to diet or a lifestyle modification programme.

***Methods***: After a systematic search and analysis of the literature, only randomised controlled trials (RCTs), which investigated the effect of insulin-sensitizing drugs on weight loss in comparison to placebo and diet and/or a lifestyle modification programme, were included. Subjects were restricted to women of reproductive age. The main outcome measure was weight loss reported in change in body mass index (BMI).

***Results***: Thirty-one trials performing the comparisons of interest were identified. Using strict selection criteria, only fourteen trials, unintentionally all but two on women with polycystic ovarian syndrome (PCOS) only, were included in the analysis. Treatment with metformin showed a statistically significant decrease in BMI compared to placebo (WMD -0.68, 95% CI -1.13 to -0.24).

***Conclusion***: This review showed that treating women of reproductive age with PCOS who are overweight or obese with metformin leads to a significant decrease in BMI, independent of the duration of treatment and addition of a diet.




## INTRODUCTION

The rising prevalence of obesity in women worldwide has implications for their reproductive outcome. Obesity is associated with menstrual disorders and anovulation (Hartz *et al*., 1979; Green *et al*., 1988; Grodstein *et al*., 1994; Lake *et al*., 1997) but fertility is also decreased in women with regular menstrual cycles who are overweight (Jensen *et al*., 1999; van der Steeg *et al*., 2008). Furthermore, women who are overweight or obese while undergoing assisted reproduction have lower pregnancy rates and higher miscarriage rates (Wang *et al*., 2000; Lintsen *et al*., 2005; Maheshwari *et al*., 2007). During pregnancy, obesity leads to a significant increase in pregnancy complications (Cedergen, 2004; Weiss *et al*., 2004) and difficulties during labour (Sebire *et al*., 2001). Infants are at greater risk of congenital abnormalities (Waller *et al*., 1994; Werler *et al*., 1996) and intra-

uterine demise (Stephansson *et al*., 2001; Nohr *et al*., 2005) contributing to an increase in perinatal morbidity and -mortality.
Obesity is characterised by insulin resistance and consequent hyperinsulinaemia. Hyperinsulinaemia contributes to anovulatory infertility by increased ovarian androgen secretion (Poretsky, 1991; Dunaif, 1997). In women with PCOS, insulin enhances intra-ovarian steroidogenesis by interacting with luteinising hormone (LH) leading to inappropriate advancement of granulosa cell differentiation and arrest of follicle growth (Franks, Robinson *et al*., 1996;Willis, Mason *et al.*, 1996).

The cornerstone of the treatment of obesity should be based on lifestyle changes by diet, exercise and behavioural modification (NIH, 1998). In obese women with anovulatory infertility, weight loss leads to spontaneous ovulation and improves the chances of spontaneous conception (Kiddy *et al*., 1992; Guzick *et al*., 1994; Clark *et al.*, 1995; Hollman *et al*., 1996). In obese women with PCOS, a minimum of 5% loss of abdominal fat is essential for the resumption of spontaneous ovulation (Huber-Buchholz *et al*., 1999). An intensive lifestyle modification programme improves the chances of spontaneous conception and conception during fertility treatment (Clark *et al.*, 1998). Pre-pregnancy weight loss can reduce the incidence of gestational diabetes (Glazer *et al*., 2004).

In view of the low success rate in achieving weight loss and even lower success rate for maintaining this weight loss, drug therapy for obesity in conjunction with the continuation of lifestyle changes is advised according to obesity guidelines (NIH, 1998). According to a randomised controlled trial, the combination of a lifestyle modification programme with drug therapy, achieves more weight loss than a lifestyle programme alone (Wadden *et al*., 2005). Orlistat and sibutramine, two approved anti-obesity drugs, should however not be used in women who anticipate conception because of lack of safety data on their use during early pregnancy.

Insulin-sensitizing drugs are not considered anti-obesity drugs even though some evidence indicates that metformin therapy might contribute to weight loss (Knowler *et al*., 2002). A systematic review confirming the effectiveness of metformin for ovulation induction in women with PCOS, could not demonstrate that metformin contributes to weight loss (Lord *et al*., 2003). Another review on the same topic did however demonstrate a contribution of metformin to weight loss (Harborne *et al*., 2003). A recent RCT comparing three ovulation induction modalities (metformin and placebo, metformin and clomiphene, clomiphene and placebo) in women with PCOS also showed more weight loss in women treated with metformin (Legro *et al*., 2007).

If metformin treatment does contribute to weight loss, treatment of women of reproductive age with obesity and infertility could improve the chances of conception. Data on the safety of metformin use in the first trimester are re-assuring (Gilbert *et al*., 2006; Lilja and Mathiesen, 2006).
The objective of this review was to assess whether treatment of women, of reproductive age who are overweight or obese, with insulin-sensitizing agents contributes to weight loss in comparison to placebo and diet and/or a lifestyle modification programme.

## METHODS

The aim of this review was to assess the effect of insulin-sensitizing drugs on weight loss in women of reproductive age who are overweight or obese. The following clinical comparisons were assessed: (1) The effectiveness of insulin-sensitizing drugs for losing weight compared to placebo, with or without a diet/lifestyle programme. (2) The side-effects and drop-out rate reported by women taking these drugs. (3) The most effective insulin-sensitizing drug for losing weight, compared to each other, with or without a diet/lifestyle modification programme.



***Data sources***
The following databases were searched for this review: Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, and EmBASE were searched up to and including August 2007. Only randomised controlled trials were included.
Main keywords used for this search were: Body weight, BMI, obesity, RCT, diet, insulin-sensitizing drugs and weight loss (See Appendix for the full list of keywords).
Hand-searching was performed on the references used in the included studies and relevant review articles were meticulously searched for related articles. Authors were contacted for missing data or questions regarding the methodology.

***Study selection***
Titles and abstracts identified through the search strategies were independently screened by two reviewers (A. Nieuwenhuis-Ruifrok, W. Kuchenbecker). Articles were discarded if they did not meet the inclusion criteria. Full text articles were obtained of studies for potential inclusion. Independent review of the trials was undertaken by the same two reviewers to assess the quality and characteristics of the studies. Differences in opinion between the reviewers on selected articles were settled by consensus.

The subjects were women of reproductive age who are overweight (BMI 25–29.9 kg/m²) or obese (BMI ≥30 kg/m²) according to accepted diagnostic criteria (NIH, 1998) and who were deemed eligible for weight loss measures. Studies that included prepubertal and postmenopausal women were excluded.

*Main intervention*: Treatment with insulin-sensitizing drugs: metformin, pioglitazone, rosiglitazone or D-chiro-inositol. Treatment with metformin was assessed in two subanalyses according to the daily dosage of ≤1500 mg or >1500 mg. This dosage cut-off for metformin was used because most initial studies used the dosage of 500 mg tablets 3 times daily (low dose) and later studies using 1000 mg twice daily or 850 mg tablets 2–3 times daily (high dose).
*Comparison interventions*: One or more of the following: Placebo only or placebo with diet advice or a lifestyle modification programme. The primary outcome measure was change in BMI in $kg/m^2$.
Secondary outcomes were drop-out rates and side effects caused by the drugs.

***Data synthesis and analysis***
Using RevMan for the analysis, measuring the effect in WMD and 95% confidence intervals (CI), data from the fourteen trials were stratified over two main interventions and subanalysis was performed on four co-interventions while still maintaining adequate numbers of patients for analysis.
For statistical analysis standard deviations (SDs) were required. Hence for all trials in which 95% CI or standard error of the means (SEM) were given, these values were converted into SDs. In two trials (Fleming *et al*., 2002; Kjotrod *et al*., 2004) more expanded calculations were needed to derive the SDs.

***Quality assessment***
Trial quality was assessed on minimisation of selection bias, performance bias, attrition bias and detection bias. Assessment of the quality of allocation concealment was graded in four categories: adequate (A), unclear (B), inadequate (C) or not given (D). Other trial characteristics assessed were differences in baseline values, non-compliance, standardised outcome measures, drop-out rate, the extent of losses to follow-up, side effects, blinding methods, and whether analysis was by intention to treat.

In the statistical analysis the weighted mean difference (WMD) was used to express the effect of each continuous outcome. Heterogeneity in the data was noted and cautiously explored using certain characteristics of the study, particularly assessments of quality. In order to examine the stability of the results in relation to a number of factors, sensitivity

analyses were performed. These analyses included quality of allocation concealment, blinding, treatment length over 8 weeks, high or low dose of metformin, inclusion BMI and ethnicity.

## RESULTS

***Search results***

The search strategy revealed thirty-one trials eligible for inclusion in this review. After studying these publications, seventeen trials were excluded (see separate reference list) and the data of the remaining fourteen trials were analysed. See Table 1 of included studies for full details of the fourteen included trials (extended version of Table 1 hosted on website of Human Reproduction Update).

The fourteen included studies randomised 649 women with 20 subjects in the smallest trials (Pasquali *et al*., 2000; Gambineri *et al*., 2004) and 143 in the largest trial (Tang *et al*., 2006).

Eleven of these trials were stated to be double-blind and two single-blind. One did not state the blinding method (Mitkov *et al*., 2006).

In three trials randomisation was performed by centre (Nestler and Jukubowicz, 1996; Kjotrod *et al*., 2004; Tang *et al*., 2006) and in three trials the randomisation was computer generated (Fleming *et al*., 2002; Vandermolen *et al*., 2001; Yarali *et al*., 2002).

Pasquali *et al.* (2000), packaged drug and placebo and labelled according to subject number. Then randomisation was performed in blocks of four.

One trial stated that it 'randomly placed' its subjects (Gambineri *et al*., 2004), two trials numbered the participants sequentially (Jakubowicz *et al*., 2001; Kocak et al., 2002). However, Kocak *et al*. (2002), randomised patients by order of admission, resulting in a quasi-randomised study. Two studies used random number tables (Kilicdag *et al*., 2005; Ortega-Gonzalez *et al*., 2005) and two (Crave *et al*., 1995; Mitkov *et al*., 2005) did not state the method of randomisation.

In four studies a power calculation was performed (Fleming *et al*., 2002; Kjotrod *et al*., 2004; Kilicdag *et al*., 2005; Tang *et al*., 2006), while the other nine did not mention a sample-size calculation. In three trials an analysis by intention-to-treat was stated to have been performed (Vandermolen *et al*., 2001; Fleming *et al*., 2002; Kjotrod *et al*., 2004).

**Table 1.** Characteristics of the included studies

| Trial | Methods | Participants (mean BMI in kg/m²) | Main intervention |
|---|---|---|---|
| Crave *et al.*, 1995 | RANDOMISED CONTROLLED TRIAL<br>METHOD OF RANDOMISATION: not stated<br>BLINDING: double blind<br>INTENTION TO TREAT ANALYSIS: no | NUMBER RANDOMISED: 24<br>INCLUSION CRITERIA: BMI > 25 kg/m², hirsutism | High-dose metformin (n=12), placebo (n=12)<br>DURATION: 4 months |
| Fleming *et al.*, 2002 | RANDOMISED CONTROLLED TRIAL<br>METHOD OF RANDOMISATION: computer generation by pharmacy in blocks of four<br>BLINDING: double blind<br>INTENTION TO TREAT ANALYSIS: yes | NUMBER RANDOMISED: 94<br>INCLUSION CRITERIA: Oligomenorrhea, PCO* detected by ultrasound | High-dose metformin (n=26), placebo (n=39)<br>DURATION: 14 weeks |
| Gambineri *et al.*, 2004 | RANDOMISED CONTROLLED TRIAL<br>METHOD OF RANDOMISATION: randomly placed<br>BLINDING: single-blind<br>INTENTION TO TREAT ANALYSIS: no | NUMBER RANDOMISED: 20 (only the data of the metformin and placebo arms were used)<br>INCLUSION CRITERIA: BMI > 28 kg/m² and waist hip ratio >0.8; PCOS: Oligomenorrhea or amenorrhea, hyperandrogenism, PCO detected by ultrasound | High-dose metformin (n=10), placebo (n=10)<br>DURATION: 6 months |
| Jakubowicz *et al.*, 2001 | RANDOMISED CONTROLLED TRIAL<br>METHOD OF RANDOMISATION: sequentially numbered, identical containers of identical drugs**<br>BLINDING: double blind<br>INTENTION TO TREAT ANALYSIS: no | NUMBER RANDOMISED: 56<br>INCLUSION CRITERIA: Oligomenorrhea, PCO detected by ultrasound, elevated free testosterone, ovulation with clomiphene citrate 150 mg | Low-dose metformin (n=28), placebo (n=28)<br>DURATION: 7-8 weeks |
| Kilicdag *et al.*, 2005 | RANDOMISED CONTROLLED TRIAL<br>METHOD OF RANDOMISATION: random number tables and assigned through consecutively numbered opaque, sealed envelopes<br>BLINDING: double blind<br>INTENTION TO TREAT ANALYSIS: no | NUMBER RANDOMISED: 30<br>INCLUSION CRITERIA: Oligomenorrhea, hyperandrogenism and/or an elevated serum testosterone level, PCO detected by ultrasound | High dose metformin (n=15), rosiglitazone (n=15)<br>DURATION: 3 months |

*Table 1 continues on next page.*

*Table 1 continued.*

| Trial | Methods | Participants (mean BMI in kg/m²) | Main intervention |
|---|---|---|---|
| Kjotrod *et al.*, 2004 | RANDOMISED CONTROLLED TRIAL<br>METHOD OF RANDOMISATION: Randomisation was performed by the hospital pharmacist, performed in blocks of four.<br>BLINDING: double-blind<br>INTENTION TO TREAT ANALYSIS: yes | NUMBER RANDOMISED: 40<br>INCLUSION CRITERIA: Criteria for overweight: BMI > 28 kg/m² , PCO detected by ultrasound, oligomenorrhea or amenorrhea and 1 out of the next 5: high level of testosteron, low SHBG level, high LH/FSH ratio, high level of fasting insulin C, hirsutism<br>NOTE: the baseline values only include the women who finished the trial | High-dose metformin (BMI >28, n=19), placebo (BMI >28, n=21)<br>DURATION: 16 weeks |
| Kocak *et al.*, 2002 | RANDOMISED CONTROLLED TRIAL<br>METHOD OF RANDOMISATION: sequential by order of admission<br>BLINDING: double blind<br>INTENTION TO TREAT ANALYSIS: no | NUMBER RANDOMISED: 56<br>INCLUSION CRITERIA: oligomenorrhea with hirsutism, hyperandrogenaemia, or ultrasound findings of PCO. | High-dose metformin (n=28), placebo (n=28)<br>DURATION: 2 cycles |
| Mitkov *et al.*, 2006 | RANDOMISED CONTROLLED TRIAL<br>METHOD OF RANDOMISATION: not stated<br>BLINDING: not stated<br>INTENTION TO TREAT ANALYSIS: no | NUMBER RANDOMISED: 30<br>INCLUSION CRITERIA: Oligomenorrhea, PCO detected by ultrasound, hyperandrogenaemia | High-dose metformin (n=15), rosiglitazone, 4 mg/day (n=15)<br>DURATION: 3 months |
| Nestler *et al.*, 1996 | RANDOMISED CONTROLLED TRIAL<br>METHOD OF RANDOMISATION: centralized randomisation process<br>BLINDING: single blind-patient blinded<br>INTENTION TO TREAT ANALYSIS: no | NUMBER RANDOMISED: 25<br>INCLUSION CRITERIA: Oligomenorrhea, hyperandrogenemia, PCO detected by ultrasound | Low-dose metformin (n=12), placebo (n=13)<br>DURATION: 4-8 weeks |
| Ortega-Gonzalez *et al.*, 2005 | RANDOMISED CONTROLLED TRIALMETHOD OF RANDOMISATION: random number tables<br>BLINDING: double blind<br>INTENTION TO TREAT ANALYSIS: no | NUMBER RANDOMISED: 52<br>INCLUSION CRITERIA: Oligomenorrhea, PCO detected by ultrasound, hyperandrogennemia, acanthosis nigricans, fasting hyperinsulinemia & fasting glucose/insulin | High-dose metformin (n=27), pioglitazone, 30 mg/day (n=25)<br>DURATION: 6 months |

*Table 1 continues on next page.*

*Table 1 continued.*

| Trial | Methods | Participants (mean BMI in kg/m²) | Main intervention |
|---|---|---|---|
| Pasquali *et al.*, 2000 | RANDOMISED CONTROLLED TRIAL<br>METHOD OF RANDOMISATION: Generated in blocks of four<br>BLINDING: double-blind<br>SINGLECENTRE<br>INTENTION TO TREAT ANALYSIS: no | NUMBER RANDOMISED: 20 (only the data of the PCOS women were used)<br>INCLUSION CRITERIA: Obese women with PCOS diagnosed by: oligomenorrhea, hyperandrogenism, PCO detected by ultrasound | High-dose metformin (n=12), placebo (n=8)<br>DURATION: 6 months |
| Tang *et al.*, 2006 | RANDOMISED CONTROLLED TRIAL<br>METHOD OF RANDOMISATION: By means of block-of-four randomisation using random tables<br>BLINDING: double blind<br>INTENTION TO TREAT ANALYSIS: no | NUMBER RANDOMISED: 143<br>INCLUSION CRITERIA: Desire to conceive, PCOS diagnosed by; oligomenorrhea or amenorrhea, PCO detected by ultrasound. All patients had normal serum prolactin levels and normal thyroid-, liver- and renal function tests. | High-dose metformin (n=69), placebo (n=74)<br>DURATION: 6 months |
| Vandermolen *et al.*, 2001 | RANDOMISED CONTROLLED TRIAL<br>METHOD OF RANDOMISATION: computer-generated in blocks of six<br>BLINDING: double blind<br>INTENTION TO TREAT ANALYSIS: yes | NUMBER RANDOMISED: 27<br>INCLUSION CRITERIA: Oligo-ovulation, hyperandrogenism<br>NOTE: all patients received CC (50 mg/d) for ovulation induction as co-intervention after the initial 7 weeks treatment period | Low-dose metformin (n=12), placebo (n=15)<br>DURATION: 7 weeks |
| Yarali *et al.*, 2002 | RANDOMISED CONTROLLED TRIAL METHOD OF RANDOMISATION: computer-generated numbers. Centralised randomization process**<br>BLINDING: double blind<br>INTENTION TO TREAT ANALYSIS: no | NUMBER RANDOMISED: 32<br>INCLUSION CRITERIA: Oligo-ovulation, hyperandrogenism, PCO on ultrasound | High-dose metformin (n=16), placebo (n=16)<br>DURATION: 6 weeks |

* PCO, polycystic ovaries.

** Information used from the Lord et al., 2003, review.

The analysis of metformin versus thiazolidinediones was included even though these three trials tested two different thiazolidinediones; rosiglitazone and pioglitazone.

The analysis comparing the duration of trials was included as the effectiveness of the drugs involved might be correlated with the duration of its use.
Five trials were included in the 'duration ≤8 weeks' arm (Nestler and Jukubowicz, 1996; Jakubowicz *et al*., 2001; Vandermolen *et al*., 2001; Kocak *et al*., 2002; Yarali *et al*., 2002), lasting 35 days to 7–8 weeks. Six trials were included in the 'duration >8 weeks' arm (Crave *et al*., 1995; Pasquali *et al*., 2000; Fleming *et al*., 2002; Gambineri *et al*., 2004; Kjotrod *et al*., 2004; Tang *et al*., 2006), lasting from 16 weeks to 6 months.

The criteria for diet or no-diet were not very strict. When mention was made of diet or lifestyle adaptations, the trial was coded as diet, even if compliance to diet was not assessed. In seven of the included studies no mention was made of a diet (Nestler and Jukubowicz, 1996; Jakubowicz, 2001; Vandermolen *et al*., 2001; Kocak *et al*., 2002; Yarali *et al*., 2002; Kilicdag *et al*., 2005; Mitkov *et al*., 2006). In two trials (Gambineri *et al*., 2004; Ortega-Gonzalez, 2005) the women were instructed not to modify their usual eating or exercise patterns during the study period, these trials were coded as 'no diet'.
In the other five trials, diet as co-intervention was implemented with a variety of criteria (Crave *et al*., 1995; Gambineri *et al*., 2004; Kjotrod *et al*., 2004; Pasquali *et al*., 2000; Tang *et al*., 2006). Pasquali *et al*. (2000) and Gambineri *et al*. (2004) placed the women on a standardised hypo caloric diet (1200–1400 kcal daily) for the 1st month, and continuing dietary treatment for the rest of the study period. Crave *et al*. (1995) placed the women on a low fat low caloric diet (1500 kcal daily with 30% fat). Kjotrod *et al*. (2004) mentioned diet and lifestyle modification without specifying the intervention. The patients in the trial of Tang *et al*. (2006) received standardised dietary advice from a research dietician aiming for a calorie reduction of 500 kcal per day.

In one of the twelve trials clomiphene citrate (CC) was used as a co-intervention (Vandermolen *et al*., 2001). In this trial all participants received CC for the first 5 days of the first cycle. With ovulation, the CC dose did not change, but with persistent anovulation, the dosage of CC was increased by 50 mg/day for the next cycle.

All studies used for this review were fully published in peer-reviewed journals. Ten of the included trials were single-centre studies (Crave *et al*., 1995; Pasquali *et al*., 2000; Jakubowicz *et al*., 2001; Fleming *et al*., 2002; Kocak *et al*., 2002; Gambineri *et al*., 2004; Kjotrod *et al*., 2004; Kilicdag *et al*., 2005; Ortega-Gonzalez *et al*., 2005; Mitkov *et al*.,

2006) and four trials were multicentre (Nestler and Jukubowicz, 1996; Vandermolen *et al*., 2001; Yarali *et al*., 2002; Tang *et al*., 2006).

Unintentionally all studies, except two (Crave *et al*., 1995; Pasquali *et al*., 2000), were found to be on women with PCOS. The diagnostic criteria for PCOS broadly followed the National Institute of Health consensus criteria (anovulation and hyperandrogenaemia with exclusion of other endocrinopathies) or the diagnostic criteria of the Rotterdam consensus meeting (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). The following criteria were used to define overweight/obesity: In three trials all women required a BMI >28 kg/m² (Gambineri *et al*., 2004; Kjotrod *et al*., 2004; Mitkov *et al*., 2006) and in one trial (Nestler and Jukubowicz, 1996) all participants required a BMI ≥27.5 kg/m$^2$. The remaining trials (Crave *et al*., 1995; Pasquali *et al*., 2000; Jakubowicz *et al*., 2001; Fleming *et al*., 2002; Kocak *et al*., 2002; Yarali *et al*., 2002; Kilicdag *et al*., 2005; Mitkov *et al*., 2006; Tang *et al*., 2006) did not specify their criteria; seven of these trials had an average BMI at baseline >28 kg/m$^2$ in both treatment arms and in three (Crave *et al*., 1995; Kilicdag *et al*., 2005; Mitkov *et al*., 2006) the average BMI in both treatment arms at baseline was >25 kg/m$^2$.

The included trials were performed on different ethnic groups and geographical locations. Trials were performed in Europe, United States of America, Turkey, Mexico, Bulgaria and Venezuela, and one study was spread over Venezuela, USA and Europe.

The mean baseline characteristics for BMI can be found in Table 2.
All women included in the trials were of reproductive age. The duration of the trials varied between 35 days and 6 months.
Heterogeneity in this review was assessed for each analysis. Performance bias (blinding) was explored; for each trial blinding methods are stated in Table 1.

***Analyses***
The analysis was structured to address three relevant clinical comparisons as mentioned in the Methods section:
(1) The effectiveness of insulin-sensitizing drugs for losing weight compared to placebo, with or without a diet/lifestyle programme.
In this comparison there were one main analysis (A) and four sub-analyses (B–E).

**Table 2.** Baseline characteristics, BMI

| Trial | Type of outcome (BMI in kg/m$^2$) | N metformin group | Metformin group | N Placebo / thiazo-lidinedione group | Placebo / thiazo-lidinedione group | P value |
|---|---|---|---|---|---|---|
| Tang *et al.*, 2006 | BMI | 69 | 37.6±5.0 | 74 | 38.9±9.5 | 0,283 |
| Fleming *et al.*, 2002 | BMI | 45 | 34.2±8.0 | 47 | 35.0±8.7 | |
| Kocak *et al.*, 2002 | BMI | 28 | 31.9±5.4 | 28 | 30.8±4.4 | NS |
| Jakubowicz *et al.*, 2002 | BMI | 26 | 31.8±1.5 | 22 | 31.7±1.4 | 0,91 |
| Ortega-Gonzalez *et al.*, 2005 | BMI | 18 | 34.1±6.8 | 17 | 32.2±4.1 | - |
| Kjotrod *et al.*, 2004 * | BMI | 17 | 32.0±3.9 | 19 | 33.7±3.5 | 0,15 |
| Yarali *et al.*, 2002 | BMI | 16 | 28.6±4.0 | 16 | 29.6±4.8 | - |
| Kilicdag *et al.*, 2005 | BMI | 15 | 26.2±5.4 | 15 | 29.3±6.2 | NS |
| Mitkov *et al.*, 2006 | BMI | 15 | 27.9±4.6 | 15 | 28.6±4.6 | - |
| Crave *et al., 1995* | BMI | 12 | 35.2±4.2 | 12 | 32.7±5.2 | - |
| Pasquali *et al.*, 2000 | BMI | 12 | 39.8±7.9 | 8 | 39.6±6.9 | - |
| Vandermolen *et al.*, 2001 | BMI | 11 | 37.6±14.3 | 14 | 38.4±8.2 | 0,146 |
| Nestler *et al.*, 1996 | BMI | 11 | 34.1±5.0 | 13 | 35.2±4.7 | |
| Gambineri *et al.*, 2004 | BMI | 10 | 37±5.9 | 10 | 37.6±4.1 | 0,276 |

Values are means±SD.
* Baseline values in this trial only include the women who finished the trial.

*(A) Metformin versus placebo (BMI) (Figure 1)*: Eleven trials with a total of 537 women compared metformin treatment with placebo (Crave *et al*., 1995; Nestler and Jakubowicz, 1996; Pasquali *et al*., 2000; Jakubowicz *et al*., 2001; Vandermolen *et al*., 2001; Fleming *et al*., 2002; Kocak *et al*., 2002; Yarali *et al*., 2002; Gambineri *et al*., 2004; Kjotrod *et al*., 2004; Tang *et al*., 2006). Of these 537 women, only the 469 women who completed the trials were included in the analysis.
Metformin treatment contributed to a significant decrease in BMI (WMD -0.68, 95% confidence interval (CI) -1.13 to -0.24, P=0.003).
Test for heterogeneity: $Chi^2$=9.35, df=10 (P=0.50). $I^2$=0%

*(B) High-dose metformin (>1500 mg/day) versus placebo (BMI) (Figure 1, upper part):* Eight trials with a total of 429 women compared high-dose metformin (>1500 mg/day) versus placebo (Crave *et al*., 1995; Pasquali *et al*., 2000; Fleming *et al*., 2002; Kocak *et al*., 2002; Yarali *et al*., 2002; Gambineri *et al*., 2004; Kjotrod *et al*., 2004; Tang *et al*., 2006). Fifty-seven women did not complete the trial and were not included in the analysis, leaving 372 women for analysis.

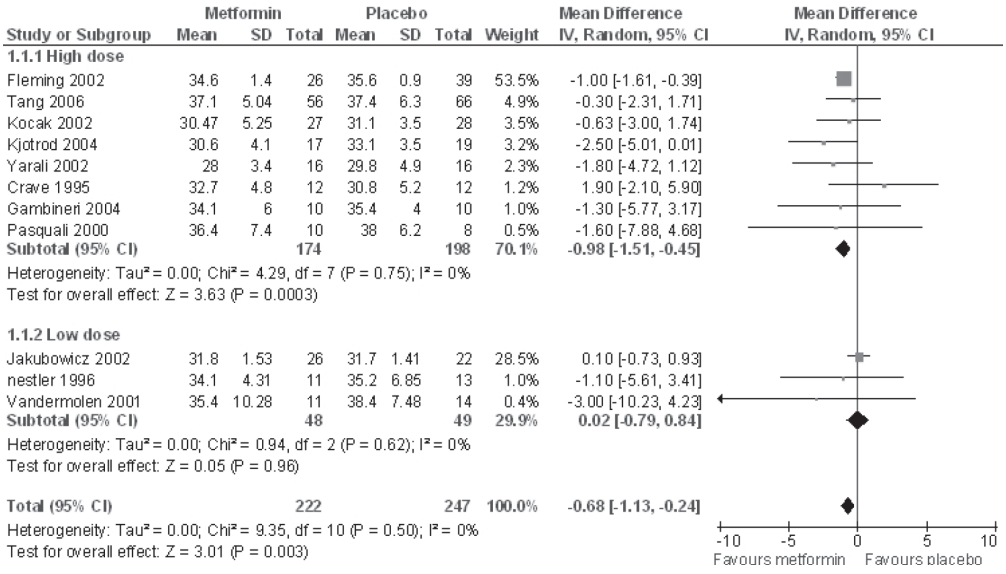


**Figure 1.** Metformin versus placebo; high dose versus low dose (BMI).

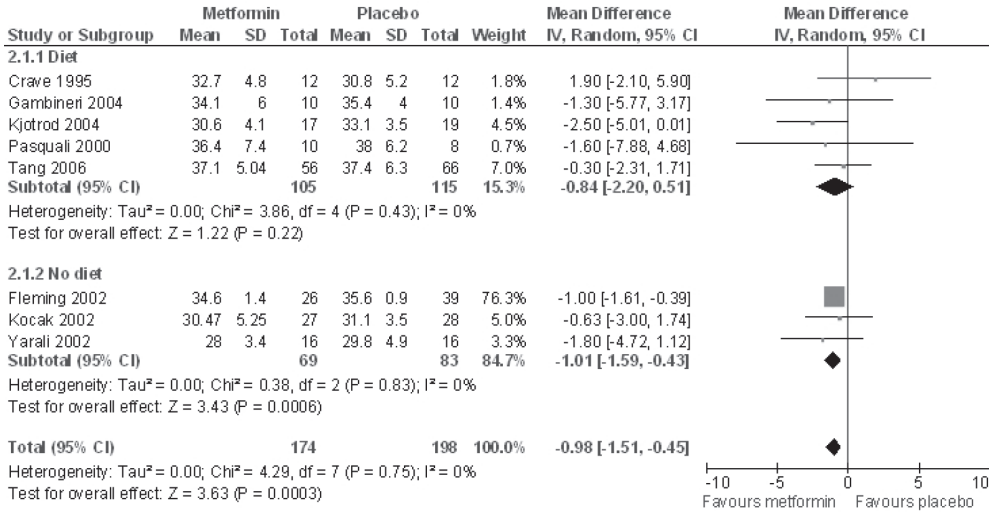


**Figure 2.** High-dose metformin versus placebo, diet versus no diet.



High-dose metformin treatment contributed to a significant decrease in BMI (WMD -0.98, 95% CI -1.51 to -0.45, P=0.003).
Test for heterogeneity: $Chi^2$=4.29, df=7 (P=0.75), $I^2$=0%.

*(C) Low-dose metformin (≤1500 mg/day) versus placebo (BMI) (Figure 1, lower part):* Three trials compared low-dose metformin (≤1500 mg/day) versus placebo, with a total of 98 women (Nestler and Jakubowicz, 1996; Jakubowicz *et al*., 2001; Vandermolen *et al.,* 2001). In this analysis 97 women were included for analysis, as one did not complete the trial.
There was no evidence of effect on BMI (WMD 0.02, 95% CI -0.79 to 0.84, P=0.96).
Test for heterogeneity: $Chi^2$=0.94, df=2 (P=0.62), $I^2$=0%.

*(D) High-dose metformin versus placebo, diet/no diet (BMI) (Figure 2):* The same trials were used as in analysis 3. Five trials included diet as a co-intervention (Crave *et al*., 1995; Pasquali *et al*., 2000; Gambineri *et al*., 2004; Kjotrod *et al*., 2004; Tang *et al*., 2006) randomising 247 women, of which 222 were included in the analyses, since 25 women did not complete the trials. Three trials in which 182 women were randomised did not include diet as a co-intervention (Fleming *et al*., 2002; Kocak *et al*., 2002; Yarali *et al*., 2002). Thirty women did not complete the trial, hence 143 were left to be analysed.

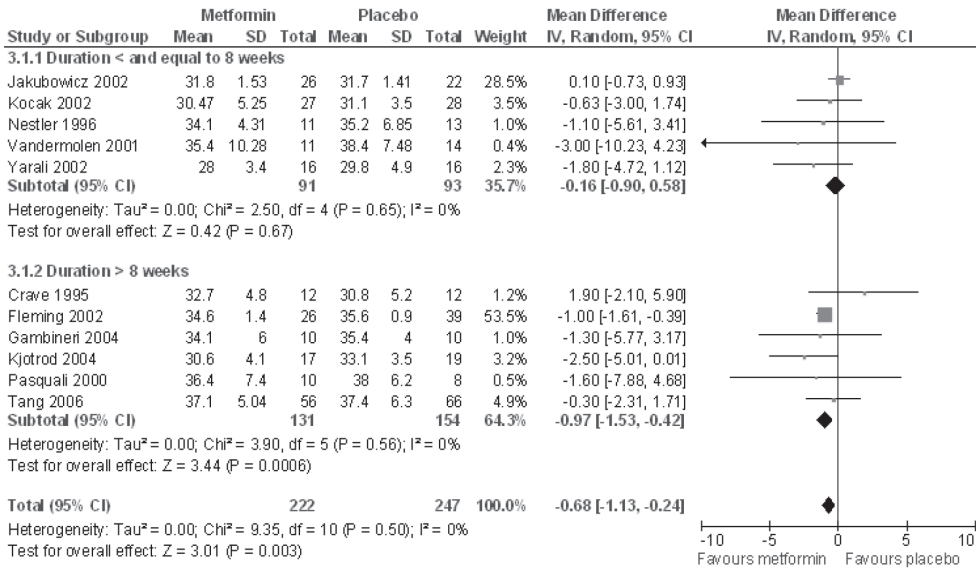


**Figure 3.** Metformin versus placebo; duration ≤8 weeks versus >8 weeks (BMI).

There was no evidence of effect on BMI in the 'diet' group, five trials of 220 women (WMD -0.84, 95% CI -2.20 to 0.51, P=0.22). In the 'no diet', three trials of 152 women, there was however a significant decrease in BMI (WMD -1.01, 95% CI -1.59 to -0.43, P=0.0006).

Test for heterogeneity (diet): $Chi^2$=3.86, df=4 (p=0.43), $I^2$=0%.

Test for heterogeneity (no diet): $Chi^2$=0.38, df=2 (p=0.83), $I^2$=0%.

*(E) Metformin versus placebo, duration ≤8 weeks/duration >8 weeks (BMI) (Figure 3):* The same trials as in analysis 1 were used; in the duration of ≤8 weeks arm five trials were included (Nestler and Jukubowicz, 1996; Jakubowicz *et al*., 2001; Vandermolen *et al*., 2001; Kocak *et al*., 2002; Yarali *et al*., 2002). In this arm 186 were randomised, of which 184 women completed the trials and were included in the analysis. In the arm of duration of >8 weeks, six trials were included (Crave *et al*., 1995; Pasquali *et al*., 2000; Fleming *et al*., 2002; Gambineri *et al.*, 2004; Kjotrod *et al*., 2004; Tang *et al*., 2006), 341 women were randomised and 287 completed the trials.

There was no evidence of effect on BMI in the 'duration ≤8 weeks', but there was evidence of effect in the 'duration >8 weeks' sub-analyses.

**Table 3**. Drop-outs and side effects

| Trial | Participants (N=) | | Side-effects reported (N=) | | Drop outs (N=) | | |
|---|---|---|---|---|---|---|---|
| | Metformin group | Placebo / thiazolidinedione group | Metformin group | Placebo / thiazolidinedione group | Total | Metformin group | Placebo / thiazolidinedione group |
| Crave *et al.*, 1995 | 12 | 12 | 8 | 1 | * | * | * |
| Fleming *et al.*, 2002 | 45 | 47 | ** | * | 29 | 21 (15) | 8 |
| Gambineri *et al.*, 2004 | 10 | 10 | 1 | * | * | * | * |
| Jakubowicz *et al.*, 2001 | 26 | 22 | * | * | 8 | 2 | 6 |
| Kilicdag *et al.*, 2005 | 15 | 15 | 3 | * | * | * | * |
| Kjotrod *et al.*, 2004 | 17 | 19 | 20 | 5 | 4 | * | * |
| Kocak *et al.*, 2002 | 28 | 28 | * | * | 1 | 1 | 0 |
| Mitkov *et al.*, 2006 | 15 | 15 | ** | * | * | * | * |
| Nestler *et al.*, 1996 | 11 | 13 | * | * | 1 | 1 | 0 |
| Ortega-Gonzalez *et al.*, 2005 | 27 | 25 | 4 | * | 17 | 9 (4) | 8 |
| Pasquali *et al.*, 2000 | 12 | 8 | ** | * | 2 | 2 | 0 |
| Tang *et al.*, 2006 | 69 | 74 | 11 | 2 | 21 | 13 (11) | 8 (2) |
| Vandermolen *et al.*, 2001 | 11 | 14 | * | * | 2 | 1 | 1 |
| Yarali *et al.*, 2002 | 16 | 16 | 1 | * | * | * | * |

*, Not stated. **, Stated, but number nor available. ( ), number between brackets represents the number of drop-outs due to side-effects.

Duration ≤8 weeks: WMD -0.16, 95% CI -0.90 to 0.58, P=0.67, duration >8 weeks: WMD -0.97, 95% CI -1.53 to -0.42, P=0.0006. Test for heterogeneity (≤8 weeks): $Chi^2$=2.50, df=4 (p=0.65), $I^2$=0%. Test for heterogeneity (>8 weeks): $Chi^2$=3.90, df=5 (p=0.56), $I^2$=0%.

(2) The side-effects and drop out rates reported by women taking these drugs.
Due to the heterogeneous description of side-effects and drop outs in the various studies we could not perform an analysis on percentages of drop outs and side-effects in the abovementioned comparisons. An overview of the side-effects and drop outs is presented in Table 3.

(3) The most effective insulin-sensitizing drug for losing weight compared to each other.
High-dose metformin versus thiazolidinedione (BMI):
Three trials with a total of 112 women compared high-dose metformin (>1500 mg/day) versus thiazolidinedione (Kilicdag *et al*., 2005; Ortega-Gonzalez *et al*., 2005; Mitkov *et al*., 2006). Ninety-five women completed the trials, only these were included in the analysis. These three trials were the only trials assessing the effect of thiazolidinedione. Therefore they were included in the analysis in spite of no placebo group.
There was no evidence of a differential effect on BMI (WMD -1.61, 95% CI -3.84 to 0.62, P=0.16).
Test for heterogeneity: $Chi^2$=0.31, df=2 (p=0.86), $I^2$=0%

***Sensitivity analyses***
Sensitivity analyses were performed on the metformin intervention arm to test whether there was an influence from allocation concealment and blinding. Excluding the three trials that were graded 'B' (Gambineri *et al*., 2004; Mitkov *et al*., 2006) or 'C' (Kocak *et al*., 2002) for allocation concealment did not change the analyses for BMI. When excluding the trials that were single blinded (Nestler and Jukubowicz, 1996; Gambineri *et al*., 2004) or did not state the blinding method (Crave *et al*., 1995; Mitkov *et al*., 2006), no change in the analyses for BMI occurred.

## DISCUSSION

The fourteen selected RCTs randomised a total of 649 women but only the 554 women who completed the trials were included for analysis. All except two of the studies analysed were on women with PCOS.

This review indicates that metformin treatment of reproductive aged women with PCOS who are overweight or obese, leads to a significant decrease in BMI when compared to placebo.
Studies assessing the treatment with high-dose metformin (>1500 mg/day) revealed a more pronounced decrease in BMI when compared to the low-dose metformin (≤1500 mg/day) studies. The fact that no significant decrease in BMI was seen in the three trials on low-dose metformin (Nestler and Jukubowicz, 1996; Jakubowicz *et al*., 2001; Vandermolen *et al*., 2001) could be attributed to shorter duration of treatment and with only 97 women analysed, this sub-analyses was potentially underpowered to show a significant effect.
Duration of the treatment for≤ 8 weeks did not show a significant decrease in BMI even after excluding the three trials on low-dose metformin (Nestler and Jukubowicz, 1996; Jakubowicz *et al*., 2001; Vandermolen *et al*., 2001).
The group with 'diet' as a co-intervention did not a show a significant decrease in BMI. The poor reporting and implementation of diets or a lifestyle programme in the analysed trials could explain this unexpected finding. On the other hand, this finding could also indicate that metformin treatment does not have an additional effect on weight loss in patients who undergo a diet or lifestyle programme.
Due to inconsistent reporting of the gastrointestinal side-effects of metformin(nausea, abdominal cramps and diarrhoea) in the trials, this review could not assess whether the significant decrease in BMI in the metformin group can be attributed to gastrointestinal side-effects. A RCT comparing different doses of metformin in obese women with PCOS (Harborne *et al*., 2005), showed that high-dose metformin (2550 mg/day) had a more consistent effect on weight loss compared to low-dose metformin (1500 mg/day) without increased drop outs rates due to gastrointestinal side effects.
Treatment with metformin did not significantly contribute to a decreased BMI when compared to thiazolidinedione. This finding was unexpected because treatment with thiazolidinedione is known to contribute to weight gain (Kahn *et al*., 2006; Balas *et al*., 2007). With only 95 women completing the trials, this analysis was potentially under-powered to show more weight loss in the metformin group.

The most important limitation of this review is that the analysis was only performed on the study subjects completing the trial period. Because only three trials (Fleming *et al*., 2002; Kjotrod *et al*., 2004; Vandermolen *et al*., 2001) used intention to treat analysis and the other trials inconsistently reported baseline BMI values and withdrawal after randomisation, sound statistical analysis was not possible using all included patients and a sensitivity analysis including and excluding the withdrawals after randomisation and the drop outs was not possible. The second limitation of this review is that most of the fourteen included trials

were designed for a different primary outcome and clinical question. Heterogeneity of the study populations is another limitation of this review.
What would the clinical relevance be of minimal additional weight loss due to high-dose metformin treatment? With the average women analysed in this review having a BMI of 35 kg/m$^2$ and assuming an average length of 1.64 m, treatment with high-dose metformin would have contributed to reach a final BMI of 34.02 kg/m$^2$ (WMD -0.98) and a decrease in weight from 94.2 kg to 91.5 kg. This decrease of 2.7 kg corresponds to a 2.9% decrease in initial bodyweight.
Orlistat and sibutramine, two of the registered anti-obesity agents, taken for a period of one year, contribute to 2.9% and 4.6% additional weight loss respectively (Padwal *et al*., 2003; (Padwal and Makumbar, 2007). Considering the lack of safety data, both of these medications should not be taken during conception and early pregnancy while data on the use of metformin during conception and early pregnancy are extensive and reassuring (Gilbert *et al*., 2006; Lilja and Mathiesen, 2006; Legro *et al*., 2007).
In overweight or obese women with PCOS a minimum of 5% weight loss is required for resumption of ovulation and spontaneous conception (Kiddy *et al*., 1992; Hollman *et al*., 1996). In obese women with PCOS, a minimum of 5% loss of abdominal fat is essential for the resumption of spontaneous ovulation (Huber-Buchholz *et al*., 1999). Minimal weight loss improves the chances of spontaneous conception and conception after fertility treatment in obese women undergoing a lifestyle modification programme (Clark *et al*.. 1998). Modest pre-pregnancy weight loss decreases the incidence of gestational diabetes (Glazer *et al*., 2004) and prevention of minimal weight gain between pregnancies decreases the chance of pregnancy complications (Villamor and Cnattingius, 2006).
With the above-mentioned evidence in mind, the 2.9% additional weight loss achieved when treating women with PCOS who are overweight or obese with high-dose metformin therapy (with a maximum intervention period in this review being 6 months) can be compared to the additional weight loss achieved with orlistat and sibutramine (with an intervention period of 1 year). High-dose metformin therapy can therefore be considered a safe and possibly relevant intervention in women with PCOS who are overweight or obese to achieve additional weight loss.

In conclusion, this review shows that treating women of reproductive age with PCOS who are overweight or obese with metformin leads to a significant decrease in BMI. Considering the limitations of this review, this conclusion should be interpreted with caution. There is some indication of greater effect with high-dose metformin (>1500 mg/day) and longer duration of therapy (>8 weeks) leading to 2.9% additional weight loss. This is however based on subgroup analysis which was probably underpowered for the low-dose and short

duration subgroups. According to this review, the addition of a diet or lifestyle programme does not contribute to further weight loss.
A structured lifestyle modification programme to achieve weight loss should still be the first line treatment in obese women with or without PCOS. Adequately powered RCTs are required to confirm the findings of this review and to assess whether the addition of high-dose metformin therapy to a structured lifestyle modification programme might contribute to more weight loss.

## APPENDIX – Full list of key words

anti-obesity agents
biguanides
body-fat distribution
body mass index/BMI
body weight
central abdominal/subcutaneous/visceral fat
D-chiro-inositol
D-chiro-inositol-galactosamine
clinical trial
controlled clinical trial
diet
double-blind
hyperandrogenism
hyperinsulinaemia
hyperinsulinism
hypoglycaemic agents
insulin-sensitizing agents
metformin
obesity
overweight
pioglitazone
placebo
random allocation
randomised controlled trial
rosiglitazone
rosiglitazone-metformin combination
single-blind
troglitazone
waist circumference
waist-to-hip ratio
weight
'weight cycling'
weight gain
weight loss
weight reduction

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# Chapter 8

# Discussion and Recommendations

# Discussion

The aim of this thesis was to assess various aspects of obesity and female infertility with the main focus on studying the role and influence of body-fat distribution, especially IAF and SAF and serum adipokines in women with obesity and infertility undergoing a lifestyle programme.

Baseline assessment of 57 women with obesity and infertility revealed that trunk fat, abdominal fat and SAF but not IAF were associated with anovulation. On the other hand, in 32 women with PCOS, early and consistent loss of IAF and not SAF was associated with resumption of ovulation. In spite of significantly more abdominal obesity and significantly more SAF and no difference in IAF in the PCOS women compared to non PCOS controls at baseline, PCOS status had no influence on the correlation of the body-fat distribution parameters with the serum adipokine levels. In women with obesity and infertility, the measurement of IAF by US and not SAF correlates strongly with the measurement by CT scan and US can measure the changes of IAF over time only with when sufficient loss of IAF has occurred.

A systematic review showed that metformin treatment of reproductive aged women with PCOS who are overweight or obese, leads to a significant decrease in BMI when compared to placebo. We have shown that the costs of fertility treatment and of pregnancy complications in obese women compared to women of normal weight, were € 6045 and € 3016 in anovulatory women and € 10,355 and € 6096 in ovulatory women per live birth, respectively.

***Obesity, adipose tissue dysfunction and body-fat redistribution***

In women of reproductive age with obesity and infertility, it is essential to identify women with abdominal obesity because of its association with infertility and pregnancy complications (Wass *et al*., 1997; Sattar *et al*., 2001; Zaadstra *et al*., 1993; Brisson *et al*. 2010). In women with abdominal obesity a further risk assessment can be performed by identifying individuals with predominantly IAF accumulation compared to those with more SAF accumulation. Little controversy exists that an excess of IAF is associated with a high cardiometabolic risk (Jensen, 2008). Subcutaneous fat on the other hand can have a protective effect against developing cardiometabolic disease by buffering postprandial free fatty acid (FFA) and lipid fluxes (Koska *et al*., 2008; Weiss, 2007). In premenopausal women with abdominal obesity, IAF is associated with IR even after correcting for SAF (Ross *et al*., 2002). In women with PCOS limited evidence indicates that an excess of IAF is associated with IR (Lord *et al*., 2006), and also with IR and increased diastolic blood

pressure during pregnancy (Bartha *et al*., 2007, Martin *et al*., 2009). We have shown that in women with obesity (BMI 37.7±6.1 kg/m$^2$) and infertility anovulatory women have a significantly higher volume of SAF and fasting insulin levels and no difference in the volume of IAF compared to ovulatory women, and after multiple logistic regression analysis, SAF and not IAF was associated with anovulation (Chapter 3). When limiting the baseline comparison of the body-fat distribution parameters to women with PCOS compared to ovulatory controls (BMI 36.8±4.9 kg/m$^2$), PCOS women had significantly more abdominal obesity (waist circumference and abdominal fat on DEXA scan) and SAF, but no difference in IAF (Chapter 5). Barber *et al*. showed that women with PCOS (BMI 28.2 kg/m$^2$) had equal amounts of IAF and significantly more SAF than ovulatory controls, but after adjusting for the differences in BMI and total fat mass, there were no significant differences in IAF and SAF anymore (Barber *et al*., 2008a). Previous studies comparing the body-fat distribution and volumes of IAF and SAF between non-obese women with PCOS to lean ovulatory controls, did not consistently show a difference in abdominal obesity and IAF (Yildirim *et al*., 2003; Dolfing *et al*., 2011; Manneras-Holm *et al*., 2011).
The discrepancy of the findings in the abovementioned studies on the volumes of IAF and SAF in women with infertility and PCOS can be attributed to the differences in BMI of the various study populations. According to the so-called 'critical intra-abdominal fat threshold' hypothesis (Freedland, 2004), with further increasing BMI and continuous excess calorie intake, IAF reaches a point of saturation after which fat is shunted to SAF. When subcutaneous adipose tissue can not store further excess fat due to adipose tissue dysfunction, a process of body-fat redistribution is initiated.
In order to increase its storage capacity of excess fat, subcutaneous adipose tissue regulates the recruitment and differentiation of preadipocytes to mature adipocytes (adipogenesis), leading to more adipocytes that can store excess fat (hyperplastic obesity) (Danforth, 2000, Rodriguez-Acebes *et al*., 2010). In many obese patients, after chronic exposure to energy dense diets and increasing BMI, adipogenesis becomes dysregulated, leading to adipocyte hypertrophy and decreased fat storage capacity of the subcutaneous adipose tissue (hypertrophic obesity) (Danforth, 2000; Villa and Pratley, 2011). Hypertrophic obesity is associated with increased fat cell size, necrosis and an inflammatory response (Rasouli and Kern, 2008; Weiss, 2007), resulting in a decreased ability to store additional fat leading to dysfunctional subcutaneous adipose tissue. Due to the dysfunctional subcutaneous adipose tissue, a process of body-fat redistribution occurs, leading to shunting of excess fat from SAF to ectopic sites like the liver, skeletal muscles and pancreas. Accumulation of fat in these ectopic sites is strongly associated with IR and an adverse metabolic profile (Weiss 2007; Koska *et al.*, 2008; Arsenault *et al*., 2011) . We hypothesize that with increasing BMI initially more fat is shunted from IAF to SAF, explaining that in women with obesity and infertility, SAF and not IAF volume is associated with anovulation (Chapter 3). This may

not be the case in women with lower BMI levels, in whom body-fat redistribution has not been initiated.
Some studies indicate that androgen excess in women with PCOS contribute to impaired adipogenesis. Dihydrotestosterone decreases the differentiation and lipid accumulation in human preadipocytes and adipocytes in culture (Gupta *et al*., 2008). Prenatal exposure to androgens in animal models induces increased adipocyte cell size accompanied by IR (Roland *et al*., 2010). We therefore hypothesize, that androgen excess in women with PCOS may contribute to impaired adipogenesis and initiate the process of adipose tissue dysfunction at a lower BMI level compared to non-PCOS women.
Based on Chapter 3 in which we show that SAF is associated with anovulation, it can not be excluded that functional mechanisms of IAF contribute to anovulation. In spite of a much smaller volume of IAF compared to SAF, and a much lower contribution of IAF to the systemic FFA concentration, various studies showed a significant correlation of IAF with IR and metabolic complications of obesity (Arsenault *et al*., 2011; Lebovitz and Banerji, 2005). IAF has higher lipolytical activity per unit fat mass, and direct drainage into the portal vein results in increased delivery of FFAs and adipokines to the liver (Weiss, 2007; Arsenault *et al*., 2011). The functional contribution of IAF to anovulation is supported by the fact that anovulatory women with obesity and PCOS need to lose IAF and not SAF for resumption of ovulation (Chapter 6) mediated by improvement of IR. It is likely that with increasing BMI and adipose tissue dysfunction in women with obesity and infertility, the redistribution of fat from IAF to SAF and ultimately to ectopic fat sites contributes to anovulation due to increased IR. In Chapter 5, women with PCOS had significantly more SAF, higher fasting insulin levels and a trend of more moderate and severe liver-fat accumulation in spite of no difference in the amount of IAF compared to non-PCOS controls. With increasing BMI and associated adipose tissue dysfunction and body-fat redistribution, it becomes even more difficult to evaluate the individual contribution of IAF and SAF to female reproduction. Future studies on the role of IAF and SAF in female reproduction should also evaluate deposition of fat in ectopic sites like liver, skeletal muscles and the pancreas.

***The role of adipokines***
Adipose tissue is a complex and highly active endocrine organ involved in the metabolism of steroid hormones and expression and secretion of various proteins and peptides, collectively called adipokines (Ahima, 2006). Apart form IR, the secretion of various adipokines by the IAF and SAF compartments may also play a role in obesity-related infertility and anovulation (Bohler *et al*., 2010; Mitchell *et al*., 2005). In view of a significantly greater amount of SAF in anovulatory women (Chapter 3), an adipokine profile -elated mechanism contributing to anovulation could also be considered. In animal

models leptin, an adipokine mainly produced by SAF, induces anovulation by direct ovarian effects (Duggal *et al*., 2000). Adipose tissue dysfunction due to hypertrophic obesity leads to a change in the profile of adipokine secretion by IAF and SAF, ectopic fat sites and other body-fat compartments, which may have an adverse effect on female reproduction (Rasouli and Kern, 2008; Villa and Pratley, 2011). Adipokines, like leptin, adiponectin, interleukin-6 (IL-6) and tumour necrosis factor α (TNFα) have been shown to influence reproductive function due to their effects on hypothalamic function and direct effects on the ovary and endometrium (Mitchell *et al*., 2005; Robker *et al*., 2009; Bohler *et al*., 2010).

Women with PCOS and obesity are known to have abdominal obesity (Pasquali *et al*., 2006; Carmina *et al*., 2009). The increase in abdominal obesity in women with PCOS compared to weight matched ovulatory controls has however not consistently been shown in women with PCOS of normal weight (Yildirim *et al*., 2003; Dolfing *et al*., 2011; Manneras-Holm *et al*., 2011). In women with PCOS, the morphology of the adipose tissue is characterised by enlarged adipocytes and low-grade inflammation of subcutaneous adipose tissue (Escobar-Morreale *et al*., 2011; Manneras-Holm *et al*., 2011). Increased gene expression of inflammatory markers of adipose tissue has been shown in obese compared to normal-weight women with PCOS (Lindholm *et al*., 2011). In obese women (BMI 34.4±4.6 $kg/m^2$) however, Lindholm *et al*. (2011) could not show a difference in the gene expression of inflammatory markers of adipose tissue between PCOS women compared to non-PCOS controls. In women with PCOS, fat cells of IAF and SAF have different lipolytical activity (Ek *et al*., 2002; Faulds *et al*., 2003) and dysregulated gene expression for insulin signalling of omental fat compared to ovulatory controls (Corton *et al*., 2007).

Based on the differences in body-fat distribution and the differences in morphology and function of adipose tissue in women with PCOS, a difference may be expected in their serum adipokine levels compared to ovulatory controls. However, in women with PCOS, leptin levels do not differ significantly from those in age and weight-matched ovulatory controls (Remsberg *et al*., 2002; Sepilian *et al*., 2006; Carmina *et al*., 2009). The positive correlation between BMI and leptin is comparable in PCOS and ovulatory women (Brzechffa *et al*., 1996). In a meta-analysis, no significant differences in the serum levels of IL-6 and TNFα between women with PCOS and ovulatory women could be shown (Escobar-Morreale *et al*., 2011). Another meta-analysis showed lower levels of adiponectin in women with PCOS compared to ovulatory controls after correcting for BMI (Toulis *et al*., 2009). Adiponectin consists of three multimers, i.e. low, medium and high molecular weight (HMW) adiponectin, of which the HMW adiponectin is specifically associated with insulin sensitivity. More recent studies did not consistently show lower HMW adiponectin levels in women with PCOS compared to ovulatory controls after correcting for differences in BMI (Barber *et al*., 2008b; O'Connor *et al*., 2010; Wickham *et al*., 2011).

We tested the hypothesis whether PCOS status is a determining factor in the correlation between body-fat distribution parameters and serum adipokine levels. Women with PCOS revealed significant differences in body-fat distribution parameters compared to non-PCOS controls (Chapter 5) and the correlation of serum adipokine levels with body-fat distribution parameters were in agreement with previous publications (Kershaw and Flier 2004; Carmina *et al*., 2009; Jain *et al*., 2009). Logistic regression analysis correcting for BMI and age however, revealed that PCOS status had no influence on the correlation (Chapter 5).

Redistribution of fat and altered function of all body-fat compartments (including ectopic sites) is reflected in a changing profile of adipokine secretion (Freedland, 2004; Weiss, 2007; Despres *et al*., 2008; Rasouli and Kern, 2008). With increasing BMI and adipose tissue dysfunction, redistribution of fat occurs including deposition of fat in ectopic sites like the liver, skeletal muscles and pancreas. With increasing BMI therefore, the total fat mass and not the individual fat compartments is predominant, explaining why PCOS status at higher BMI levels is not a determining factor in the correlation between body-fat distribution and serum adipokine levels.

On the other hand, serum adipokine levels may not accurately reflect the morphology and function of adipose tissue compartments for the following reasons. Some adipokines (like adiponectin) are exclusively secreted by adipocytes, while only one third of circulating IL-6 originates from adipocytes. Leptin on the other hand is also expressed by other cells within the fat depots and by other tissues. TNFα acts primarily in an autocrine and paracrine manner and its serum levels therefore may not reflect its biological activity (Rasouli and Kern, 2008; Bohler *et al*., 2010). Serum levels of adipokines do not accurately reflect the release of adipokines by IAF into the portal venous system in spite of the known metabolic consequences of hepatic fat accumulation (Weiss, 2007; Arsenault *et al*., 2011). At present it is not possible to define the contribution of ectopic fat depots to serum adipokine levels. The mere measurement of the volumes of IAF and SAF and serum adipokine levels will not accurately reflect the metabolic activity of the different fat compartments, let alone their effect on female reproduction. Studies on the effect of obesity on female reproduction by analysing serum adipokine levels should take these limitations into consideration. The measurement of serum adipokine levels should not be used in the clinical management of women with obesity and infertility.

In Figure 1 an overview is presented of the concept of adipose tissue dysfunction and of the proposed mechanisms by which accumulation and dysfunction of intra-abdominal adipose tissue and subcutaneous adipose tissue can be linked to the metabolic and female reproductive consequences of obesity. In Figure 1 it is also explained why women with hyperplastic obesity can maintain a metabolically healthy obese phenotype.

**Figure 1.**

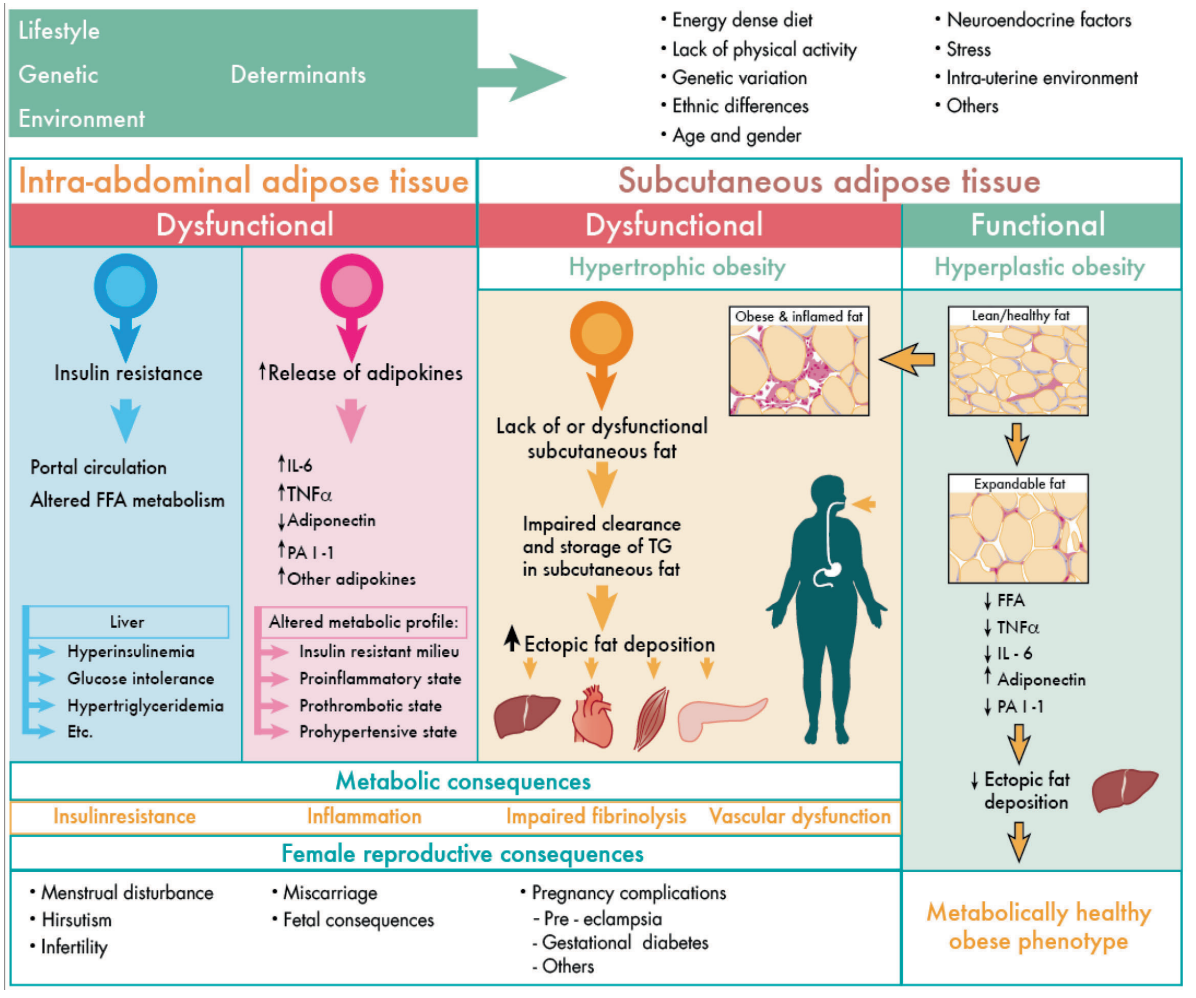
Lifestyle
Genetic
Environment
Determinants
• Energy dense diet
• Lack of physical activity
• Genetic variation
• Ethnic differences
• Age and gender
• Neuroendocrine factors
• Stress
• Intra-uterine environment
• Others
Intra-abdominal adipose tissue
Dysfunctional
Subcutaneous adipose tissue
Dysfunctional
Functional
Hypertrophic obesity
Hyperplastic obesity
Insulin resistance
Portal circulation
Altered FFA metabolism
↑Release of adipokines
↑IL-6
↑TNFα
↓Adiponectin
↑PA I -1
↑Other adipokines
Liver
Hyperinsulinemia
Glucose intolerance
Hypertriglyceridemia
Etc.
Altered metabolic profile:
Insulin resistant milieu
Proinflammatory state
Prothrombotic state
Prohypertensive state
Lack of or dysfunctional subcutaneous fat
Impaired clearance and storage of TG in subcutaneous fat
Ectopic fat deposition
Obese & inflamed fat
Lean/healthy fat
Expandable fat
↓ FFA
↓ TNFα
↓ IL - 6
↑ Adiponectin
↓ PA I -1
↓ Ectopic fat deposition
Metabolic consequences
Insulinresistance
Inflammation
Impaired fibrinolysis
Vascular dysfunction
Female reproductive consequences
• Menstrual disturbance
• Hirsutism
• Infertility
• Miscarriage
• Fetal consequences
• Pregnancy complications
- Pre - eclampsia
- Gestational diabetes
- Others
Metabolically healthy obese phenotype

**Figure 1.** Proposed mechanisms by which accumulation of intra-abdominal and subcutaneous adipose tissue can cause dysfunctional and functional obesity, and be linked to metabolic and female reproductive consequences of obesity. (Adapted with permission from (Despres *et al.*, 2008.)
FFA, free fatty acids; IL-6, interleukin-6; TG, triglycerides; PAI-1, plasminogen activator inhibitor-1; TNFα, tumour necrosis factor α.

***Weight loss for the improvement of female reproductive outcome***

Weight loss and exercise in women who are overweight or obese improve the chances of spontaneous conception in anovulatory and ovulatory women (Clark *et al*., 1998; Norman *et al*., 2004) and a decrease in BMI before conception leads to the improvement of pregnancy outcomes (Glazer *et al*., 2004; Villamor and Cnattingius, 2006; Paramsothy *et al*., 2009). According to a recent systematic review and meta-analysis, dietary and lifestyle intervention even during pregnancy can improve perinatal and obstetric outcomes (Thangaratinam *et al*., 2012). Weight loss interventions for the improvement of reproductive outcome in women with obesity and infertility are based on retrospective and small cohort studies, and have not yet been evaluated in randomised controlled trials.

Previous studies have shown that 5–10% loss of body weight leads to resumption of ovulation in about 60% of anovulatory women who are overweight or obese (Kiddy *et al*., 1992; Guzick *et al*., 1994; Clark *et al*., 1995), and that 5% loss of abdominal fat on DEXA is associated with resumption of ovulation in overweight women with PCOS (Huber-Buchholz *et al*., 1999). We have shown that obese anovulatory women with PCOS who resume ovulation during a 6-month lifestyle programme lose 15% abdominal fat on DEXA compared to 4.3% in women who did not resume ovulation. Furthermore, women with PCOS who resume ovulation lost significantly more IAF, with no difference in the loss of SAF compared to women who did not resume ovulation (Chapter 6). As shown in different patient populations during the initial period of calorie restriction preferential loss of IAF occurs, which significantly correlates with an improvement in IR (Goodpaster *et al*., 1999). Surgical removal of IAF leads to improvement in IR (Thorne *et al*., 2002), while liposuction does not alter IR significantly (Klein *et al*., 2004). In anovulatory women with obesity achieving weight loss, resumption of ovulation is mediated by improvement in IR and a decrease in free androgen levels (Guzick *et al*., 1994; Holte *et al*. 1995; Pasquali *et al*. 2006). Improvement in IR and the resulting lower insulin levels lead to less androgen production by the ovarian theca cells and more SHBG production in the liver (Poretsky, 1991), and the lower free androgen levels further contribute to the resumption of ovulation (Pasquali *et al*., 2003; Guzick *et al*., 1994).

In Chapter 6, there was a non-significant increase in pedometer steps (an indication of increased physical activity) in the women that resumed ovulation compared to those that remained anovulatory during the lifestyle programme. It was not an aim of this study to

assess whether increased physical activity may contribute to increased loss of IAF and resumption of ovulation. Some studies suggest that exercise contributes to loss of IAF (Kay and Fiatarone Singh, 2006; Ohkawara *et al*., 2007). A study controlling for calorie intake and diet could not show an independent effect of exercise on loss of IAF (Christiansen *et al*., 2009), but a more recent study indicated that exercise was associated with loss of IAF and improvement in IR in PCOS women, despite weight maintenance (Hutchison *et al*., 2011). A structured exercise programme in anovulatory women with PCOS showed higher ovulation rates and improvement in IR compared to a diet programme alone (Palomba *et al*., 2008). Data prospectively collected from the Nurses' Health Study II reveal that for every hour of vigorous physical activity per week, there was a 5% reduction in the relative risk of ovulatory infertility after correcting for BMI (Rich-Edwards *et al*., 2002). In a recent prospective cohort study of physical activity and time to pregnancy, moderate physical activity was associated with a small increase in fecundability regardless of BMI (Wise *et al*., 2012). In women of normal weight, a decrease in fecundability with vigorous physical activity was seen in a dose-response relationship (Wise *et al*., 2012). In women with overweight or obesity however, a weak positive association was seen between vigorous activity and fecundability (Wise *et al*., 2012). Overweight or obese women are more IR and vigorous physical activity may improve glucose disposal and IR by increasing the muscle mass (Carroll and Dudfield, 2004; Thomson *et al*., 2008), while in normal-weight women vigorous physical activity alters the GnRH pulse activity contributing to avulatory dysfunction.

Most individuals who are overweight or obese experience great difficulty to achieve and maintain weight loss, and a multi-factorial approach based on diet, exercise and behaviour modification is advised to help patients lose weight (Anonymous, 1998). Dietary interventions should be tailored to individual preferences aimed at achieving a 600 kcal/day deficit. Generally, obese individuals achieve maximal weight loss after the first 6 months of weight loss intervention irrespective of the dietary composition (Sacks *et al*., 2009). According to a meta-analysis, exercise in combination with dietary intervention achieves more weight loss than diet alone (Wu *et al*., 2009), and it has a favorable effect on general well-being and health-related quality of life (Lemoine *et al*., 2007). Obese individuals have an intrinsic resistance to changing behaviour, and low self-esteem and low self-efficacy are a limiting factor in achieving weight loss. Individual or group support and cognitive-behavioural guidance are advised and will contribute to more and sustained weight loss (Wadden and Butryn, 2003; Shaw *et al*., 2005; Sacks *et al*., 2009). It is essential to try to achieve adequate weight loss soon after the start of a lifestyle programme, because too little weight loss increases the risk of drop-out (Messier *et al*., 2010). In our study, in spite of personal guidance by a nurse practitioner using motivational interviewing techniques, the drop-out rate in the lifestyle programme in women with obesity and infertility was 30%.

This is in agreement with the rates of 27–35% reported in previous studies on lifestyle intervention in comparable patient populations (Clark *et al*., 1995; Hoeger *et al*., 2004; Palomba *et al*., 2008). Drop-out in women with obesity and infertility undergoing a lifestyle programme is a major limiting factor in achieving the maximal benefit of weight loss on female reproductive outcome. More studies should be performed to elucidate the patient-related factors that lead to high chances of drop-out.

According to several international guidelines, women with overweight and obesity are required to lose weight before conception. Some fertility clinics even expect women with severe obesity to reduce their BMI to <35 kg/m$^2$ in order to limit serious obesity-related pregnancy complications (Balen *et al.*, 2006; Nelson and Fleming, 2007). Women with severe obesity who are unable to reduce their weight sufficiently are faced with not being accepted for fertility treatment (Farquhar and Gillett, 2006; Pandey *et al*., 2010). A national survey in the US indicated that in spite of the absence of firm guidelines on BMI restrictions, most fertility specialists believe that BMI guidelines or cutoffs should exist (Harris *et al*., 2011). In women with infertility and severe obesity who have failed to lose adequate weight during a lifestyle programme, it is essential to consider additional treatment options like weight loss medication and bariatric surgery. These interventions may help them to reduce their BMI and to ensure their access to fertility treatment.

The combination of a lifestyle modification programme with weight loss medication, achieves more weight loss than a lifestyle programme alone (Wadden *et al*., 2005). The effect of weight loss medications is however modest and they are limited by side effects. Orlistat, an approved anti-obesity drug should not be used in women who anticipate conception because of lack of safety data on its use during early pregnancy. The pharmacokinetics of orlistat, however, are favourable because of a very low absorption and first-pass metabolism, resulting in a bioavailablity of less than 1% (Padwal and Majumdar, 2007). Further studies are needed before sound recommendations can be made regarding the use of orlistat in women attempting to conceive.

Insulin-sensitizing drugs are not considered weight loss medications, even though some evidence indicates that metformin therapy might contribute to weight loss (Knowler *et al*., 2002). Not all studies on the use of metformin in women with PCOS could show that metformin use contributes to weight loss *(*Harborne *et al., 2003;* Lord *et al., 2003;* Legro *et al*., 2007). In anovulatory women with PCOS, metformin was studied extensively and may contribute to resumption of ovulation in a subset of patients (Tang *et al*., 2012). We performed a systematic review on the use of insulin-sensitizing drugs for weight loss, expressed as change in BMI, in comparison to placebo and diet and/or a lifestyle modification programme in women of reproductive age. Treatment with metformin showed a statistically significant decrease in BMI compared to placebo (weighted mean difference -0.68, 95% CI -1.13 to -0.24) (Chapter 7). Treatment with high-dose metformin (>1500

mg/day) revealed a more pronounced decrease in BMI when compared to low-dose metformin (≤1500 mg/day), but treatment for less than 8 weeks did not show a significant decrease in BMI even after excluding trials on low-dose metformin. Data on the safety of metformin use in the first trimester of pregnancy are re-assuring (Gilbert *et al*. 2006; Lilja and Mathiesen, 2006). In women with infertility and severe obesity who have great difficulties achieving sufficient weight loss, treatment with high-dose metformin for more than 8 weeks may help them to achieve more weight loss and reach a target BMI in order to be considered for fertility treatment in fertility units that maintain BMI entry criteria.

Women with a BMI >40 kg/m$^2$ who can not reach sufficient weight loss, have to be informed about the option of bariatric surgery. Bariatric surgery leads to significant weight loss up to 5 years, with significant improvement and even resolution of type 2 diabetes mellitus, hypertension and hypertriglyceridaemia (Padwal *et al*., 2011). Bariatric surgery leads to significant improvement in obesity-related pregnancy complications, especially a decrease in gestational diabetes and pre-eclampsia (Maggard *et al*., 2008; Kominiarek, 2010). The three most common bariatric surgery procedures are laparoscopic Roux-en-Y gastric bypass (LRYGB), laparoscopic sleeve gastrectomy (LSG) and laparoscopic adjustable gastric banding (LAGB). LRYGB leads to a higher degree of weight loss than LAGB (68% vs. 45% of preoperative weight after 4 years), and weight loss after LRYGB and LSG is comparable (Colquitt *et al*., 2009; Padwal *et al*., 2011). LAGB has less short term complications, but a higher rate of later re-operation than LRYGB due to band migration or erosion and inadequate weight loss. It is recommended that contraception should be used during the first 12 to 18 months after the operation, to benefit from maximal weight loss and to avoid the fetus being exposed to a maternal environment of rapid weight loss that may predispose to poor fetal growth (American College of Obstetricians and Gynecologists, 2005; Lesko and Peaceman, 2012).
Data on female fertility after bariatric surgery are limited, but indicate that the amount of weight loss is the most important predictor of spontaneous conception (Musella *et al*., 2011). After bariatric surgery, the nutritional status of the pregnant women should be optimised and iron, folic acid and vitamin B12 supplementation is advised. These women require special attention and care during pregnancy by a multi-disciplinary team because of the risk that late operative complications may be mimicked by pregnancy (Wax *et al*., 2007).

## METHODOLOGICAL CONSIDERATIONS

***Sample size and repeat measurements***

All infertile women with a BMI >29 kg/m$^2$ attending the fertility clinic of the University Medical Center Groningen were considered for inclusion in a lifestyle intervention programme of 6 months. Of the 60 eligible women who agreed to participate, two did not undergo the baseline assessment due to early drop-out and pregnancy. One anovulatory woman with a BMI of 58 kg/m$^2$ was excluded from the analysis because the CT could not be performed accurately due to physical constraints. See Table 3 of the introduction for a summary on how the study subjects were selected for the analyses of Chapters 3 to 6. The number of participants decreased due to drop-out and pregnancy (11 dropped out and six became pregnant during the first 3 months, and seven dropped out and six became pregnant during the last 3 months) of the lifestyle programme. The resulting small numbers of participants is a limiting factor for the analyses in Chapters 3 to 6. Small sample size may reduce statistical power and may be influenced by outlying values, but scatter plots did not reveal outlying values. Repeated measurements on the same subjects in this study revealed consistent and significant results. This indicates that the inter-subject variation between the different measurements was limited, which mitigates the effect of the small numbers on the analyses.

Incomplete data due to drop-out in lifestyle intervention studies may compromise the longitudinal analysis of the data on the outcome of lifestyle intervention, because the number of subjects available for complete case analysis will be reduced (Ware, 2003). Various statistical methods are used in an effort to make maximum use of all available data (Liu and Gould 2002; Gadbury *et al*., 2003). In the analysis of the change of IAF in anovulatory women (Chapter 6), we started with a complete case analysis using ANOVA which showed no significant differences but a trend of the findings. To analyse the trend, we performed Generalised Estimating Equations (GEE) in a longitudinal design making maximum use of the available data by including the data of the last measurement before drop-out or pregnancy.

***Validation between US and CT measurements of IAF and SAF***

IAF and SAF are measured by US as a distance in cm and by CT as a volume in cm$^3$. The validation between US and CT measurements of IAF and SAF can not be determined because of the different measurement units. We therefore applied the Bland-Altman method comparison analysis to test the relative validity by comparing the distance measurement of IAF by US and CT in cm. The findings of Chapter 4 (i.e., the US measurement of IAF correlates with the CT measurement) can be applied in future studies on the role of IAF in female reproduction. On an individual clinical patient basis, US measurement of IAF in cm

can however not replace CT measurement of IAF in $cm^3$ due to the lack of studies proving the absolute validity between US and CT measurement of IAF. However, considering the studies showing good relative validity, US measurements of IAF can be used in epidemiological research investigating the role of this adipose tissue compartment in female reproductive function.

To limit the radiation exposure in women with obesity and infertility, we used a single-sliced abdominal CT scan at the level of L4-L5 to capture the IAF and SAF distribution. Due to the individual variation of IAF and SAF distribution throughout the abdomen, whole abdominal CT or MRI measurement of the total volume of IAF and SAF is more accurate than measurement by a single-sliced abdominal CT scan at the level of L4-L5 (Thomas and Bell, 2003; Lee *et al*., 2004). Most previous validation studies also used CT or MRI slices at the level of L4-L5 or L2-L3, and their findings are in agreement with our data obtained at L4-L5 (Armellini *et al.*, 1993; Stolk *et al*., 2001; De Lucia Rolfe *et al*., 2010; Gradmark *et al*., 2010). A study published after our study was launched (Kuk *et al*., 2010), indicates that in women the measurement of IAF by single-sliced CT scan at the level of L2-L3 shows better correlation with whole abdomen CT scan. Future validation studies on the measurement of IAF between CT and US in women should use the IAF measurement of a single-sliced CT scan at the level of L2-L3.

# Recommendations

***Implications for clinical practice***

Women with infertility who are overweight or obese are often prepared to conceive at all costs with little consideration for the associated decrease in live birth rates and serious risks during pregnancy and for the future child. Fertility doctors on the other hand are obliged to discuss these risks with their patients, but have the professional responsibility to provide good quality care and to decide whether access to fertility treatment is justified. We aim to formulate critical reasoning and arguments which can be used by fertility doctors in the counselling, decision making and treatment of women with infertility who are overweight or obese.

***General advice***

All patients who are overweight or obese should be informed about the long term obesity-related health risks such as cardiovascular disease, type 2 diabetes and several cancers. Women with infertility who are overweight or obese should be informed about the detrimental effect of overweight or obesity on female fertility and treatment outcome (i.e. lower chance of pregnancy and increased risk for miscarriage). The women should specifically be counselled on the serious obstetrical risks (e.g., hypertensive disorders and pre-eclampsia, gestational diabetes, thromboembolic disorders and increased caesarean delivery rates), perinatal risks (e.g., birth complications due to macrosomia, perinatal death, neural tube defects and cardiovascular anomalies) and the long-term health of the offspring (i.e. adult disease due to unfavourable fetal conditions). These women should be counselled that weight loss before conception may improve the live birth rate and will decrease the obstetric and perinatal risks associated with overweight and obesity (Balen *et al*., 2007, Nelson and Fleming, 2007).

See Table 2 of the introduction for an overview of the effect of obesity on female reproduction.

The following arguments can be used in the counselling and decision making process, based on the individual BMI level of the patient.

***BMI 25–29.0 kg/m$^2$***

Women with WHO II anovulation should be informed that generally weight loss leads to resumption of ovulation and is therefore considered the first line of fertility treatment. Weight loss can be achieved by calorie reduction, and the addition of moderate to vigorous

exercise increases the chance of resumption of ovulation. Ovulation induction should only be considered if more than 5% loss of body weight did not lead to resumption of ovulation. Women with overweight should be advised to lose weight before starting fertility treatment in order to improve the live birth rate and decrease the obesity-related pregnancy risks. Only the women that do not achieve any weight loss should be offered a structured lifestyle programme as mentioned below.

***BMI 30–34.9 kg/m$^2$***

Women with obesity and infertility should be advised to lose weight and be offered assistance in doing so. Preferably, fertility clinics should offer a structured lifestyle programme with individual guidance and a combination of diet, increased physical activity and behaviour modification based on evidence based guidelines (National Heart, Lung and Blood Institute/National Institutes of Diabetes and Digestive and Kidney diseases, 1998). Patients should be motivated to lose 5–10% of the initial body weight in order to increase the live birth rate and decrease the obesity-related pregnancy risks. This focused effort of lifestyle intervention should be achievable in a 6 months period.

***BMI 35–39.9 kg/m$^2$***

Women with severe obesity and infertility should be informed about the serious obesity-related maternal and perinatal risks. The fertility doctor should aim to convey this message in a sympathetic manner while on the other hand convincing the patient that fertility treatment above a BMI level of 35 kg/m$^2$ will not be honoured. These women should be encouraged to participate in a structured lifestyle programme as mentioned above. The women should be informed that any delay in fertility treatment is in the best interest of the mother and the potential offspring. Women that are not able to achieve sufficient weight loss in spite of strict compliance to the lifestyle programme can be offered weight loss medication in order to achieve the target BMI level. The use of contraception should be advised during the use of orlistat. In selected patients with severe obesity who are unable to reach the target BMI of 35 kg/m$^2$ and especially those women with type 2 diabetes mellitus and hypertension, bariatric surgery can be considered as mentioned below.

***BMI ≥40 kg/m$^2$***

Women with morbid obesity should be counseled about the very serious obesity-related pregnancy risks for both mother and offspring and that no fertility treatment will be performed above a BMI level of 35 kg/m$^2$. These women should be assessed in a multi-disciplinary setting and advised on weight loss medication in addition to a structured lifestyle programme as mentioned above. In many of these women with morbid obesity, reaching a BMI level of 35 kg/m$^2$ is an unrealistic target. These women should be counseled about the effects, risks and consequences of bariatric surgery with the aim to lose sufficient

weight and achieve the target BMI level. After bariatric surgery, contraception should be advised during the period of rapid weight loss (12–18 months) as this period of metabolic instability might also have a detrimental effect on pregnancy outcome, especially the risk of intra-uterine growth retardation. After bariatric surgery, enough time should be allowed before conception, to achieve sufficient weight loss in order to facilitate the decrease in obesity-related pregnancy complications.

***Obesity vs. age***

In women above the age of 35 years, age has been shown to have a stronger negative effect on fertility than BMI (Sneed *et al*., 2008; Luke *et al*., 2011). The gain associated with weight loss should be balanced against increasing age and decreasing fertility during the months of lifestyle intervention. In women above the age of 35 years and a BMI between 35–39.9 kg/m$^2$, the severe obesity-related obstetrical and perinatal risks justify several months of structured lifestyle intervention to achieve sufficient weight loss in order to decrease these risks. The morbid obese women with a BMI ≥40 kg/m$^2$ should not be offered fertility treatment, even if they are approaching the end of their reproductive period.

***Pregnancy***

During pregnancy, weight gain should be restricted according to pre-pregnancy BMI levels (Table 1) (Rasmussen *et al*., 2010), and lifestyle intervention and dietary intervention should be encouraged in order to decrease obesity-related pregnancy complication rates (Hui *et al*., 2012; Thangaratinam *et al*., 2012).

***Ethical considerations***

An extensive deliberation of the ethical considerations of lifestyle-related factors and access to medical assisted reproduction was published by the European Society of Human Reproduction and Embryology (ESHRE) Task Force on Ethics and Law (ESHRE Task Force on Ethics and Law, including *et al*., 2010).The following ethical considerations pertaining to overweight and obesity and female infertility should be considered:

**Table 1.** Recommendations for weight gain during pregnancy (adapted from Rasmussen *et al*., 2010)

| Prepregnancy BMI | Classification | Total weight gain (range in kg) |
|---|---|---|
| BMI <18.5 | Underweight | 12.5–18 |
| BMI 18.5–24.9 | Normal weight | 11.5–16 |
| BMI 25–29.9 | Overweight | 7.0–11.5 |
| BMI ≥30 | Obese | 5.0–9.0 |

Abbreviations: Body mass index (BMI) as kg/m$^2$.

The primary aim of fertility treatment is to achieve the birth of a healthy child without exposing the future mother nor her offspring to dis-proportional risks. Professionals involved in fertility care have responsibilities for the welfare of their infertile patients and the off-spring. When considering fertility treatment, the fertility doctor has to balance the maternal risks involved and the welfare of the future child, and in case of a negative ratio of expected benefit and harm the ethical principle of non-maleficence ("first do no harm") is violated, and fertility treatment should not be offered. When fertility treatment is collectively funded and costs related to maternal and neonatal (long-term) complications are covered by public health insurance, the fertility doctor has to take the interests of society into consideration as well. Doctors and society are allowed to require patient commitment and compliance with lifestyle recommendations that will decrease the risks for the future mother and child. Withholding fertility treatment in women with severe obesity (BMI $\geq$35 kg/m$^2$) is therefore ethically justified, but fertility clinics and medical professionals offering reproductive assistance to women with obesity have the ethical obligation to assist these women to lose weight by offering or facilitating a structured lifestyle intervention programme.

## RECOMMENDATIONS FOR FUTURE RESEARCH

Besides BMI level, other body-fat distribution parameters, US measurement of IAF and liver-fat accumulation should be recorded in women with overweight and obesity considering fertility treatment. These parameters should be correlated with live birth rates and pregnancy complications in order to identify prognostic factors which can be used to select women with obesity-related poor reproductive outcome. These data may identify more specific BMI levels and body-fat distribution parameters that can be implemented for targeted lifestyle intervention before starting fertility treatment with the aim to improve reproductive outcome.

Lifestyle intervention programmes for women with infertility who are overweight or obese should be combined with studies assessing the mechanisms and effects of lifestyle intervention on female reproduction:

- A current multicenter RCT (LIFESTYLE study) is underway to assess the costs and effects of a structured lifestyle programme in overweight and obese subfertile women to reduce the need for fertility treatment and improve reproductive outcome (Mutsaerts *et al*., 2010) (Dutch Trial Register NTR1530). The findings of this study will hopefully provide guidance to clinicians when considering treatment of women with obesity and infertility

- For anovulatory women who are overweight or obese, different dietary compositions and different exercise programmes for targeted loss of IAF for resumption of ovulation should be assessed. The additional benefit of aerobic exercise with or without resistance training on resumption of ovulation should be evaluated in a randomised trial.
- The effect of the lifestyle intervention on the changes in IAF, body-fat redistribution and accumulation of fat in ectopic sites should be investigated by using US as a measurement tool of IAF according to a strict measurement protocol. Quantitative hepatic fat assessment by US can be used in a research setting to measure liver-fat accumulation as a marker of ectopic fat accumulation.
- Future studies should identify risk factors for drop-out during lifestyle intervention programmes. The outcomes of such studies can lead to individualised targeted intervention with the aim of decreasing the drop-out rate as a limiting factor in the success of lifestyle intervention programmes.

Future studies should investigate the pathofysiological mechanisms of dysfunctional adipose tissue leading to fat redistribution with accumulation of fat in ectopic sites. These studies should combine the assessment of body-fat compartments, ectopic site fat accumulation and gene expression profile assessment of omental fat and the different subcutaneous fat regions. Genome-wide microarray analysis of the different fat compartments can identify candidate genes with abnormal expression involved in the evolution of adipose tissue dysfunction related to obesity (Rodriguez-Acebes *et al*., 2010).

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# Chapter 9

# Summary and Samenvatting

# Summary

The female reproductive consequences of the rising prevalence of overweight (BMI 25–29.9) and obesity (BMI≥30) world -wide are of great concern, and amongst other disciplines also require gynaecologists to prioritise this chronic disease in clinical management protocols and research. In women with obesity, the chances of conceiving spontaneously are decreased and the risk of a miscarriage is increased. More women with obesity will therefore require fertility treatment, while these patients require more gonadotrophins for ovarian hyperstimulation and have lower pregnancy rates and live birth rates after ART. The obesity-related pregnancy complications are of great concern because of an increase in perinatal mortality and even in maternal mortality in cases of severe (BMI 35–39.9 kg/m$^2$) and morbid (BMI ≥40 kg/m$^2$) obesity. Obesity in pregnancy also predisposes to long-term disease of the offspring due to the concept of developmental onset of health and adult disease.

The studies in this thesis were undertaken to assess various aspects of obesity and female infertility. The main focus was on the role and influence of body-fat distribution, especially intra-abdominal fat (IAF) and subcutaneous abdominal fat (SAF) in women with obesity and infertility undergoing a lifestyle program.

Considering the above-mentioned consequences of obesity in women undergoing fertility treatment, it is likely that obesity has a negative impact on the cost-effectiveness of fertility treatment. In **Chapter 2** a framework was developed to calculate the costs of fertility treatment (i.e., ovulation induction, intra-uterine insemination and in vitro fertilisation) and of pregnancy complications for ovulatory and anovulatory women separately, in a hypothetical cohort of 1000 normal weight, overweight and obese women each. The calculated costs per live birth in obese women compared to women of normal weight were € 6045 and € 3016 in anovulatory women, and € 10,355 and € 6096 in ovulatory women, respectively. Future prospective studies are awaited to determine whether lifestyle intervention aimed at weight loss before fertility treatment will improve the cost-effectiveness of fertility treatment per live birth in women with obesity and infertility.

Obesity is a heterogeneous condition in which accumulation of fat around the abdomen (abdominal obesity) is associated with a high cardio-metabolic risk profile and adverse female reproductive outcome. Abdominal obesity in women of reproductive age is associated with infertility and an increase in pregnancy complications. In individuals with abdominal obesity, accumulation of IAF and not SAF is associated with insulin resistance

(IR) and a high cardio-metabolic risk profile. Limited evidence indicates that in abdominal obesity the excess of IAF has a detrimental effect on female reproduction. In **Chapter 3** we assessed the contribution of different body-fat distribution parameters (and especially IAF and SAF volume measured by abdominal Computerised Tomography (CT) scan) to anovulation in women with obesity and infertility. In spite of comparable BMI, anovulatory women had a higher waist circumference, significantly more trunk and abdominal fat on DEXA scan and significantly more SAF than ovulatory women. The volume of IAF was not significantly different between the ovulatory and anovulatory groups. After multiple logistic regression analysis, only trunk fat, abdominal fat and SAF were associated with anovulation. This association is however only based on the volume of these fat compartments and does not necessarily reflect the consequences of their metabolic activity.

Reliable measurement of IAF and SAF in women with obesity and infertility is essential for adequate evaluation of the effect of these fat compartments on female reproductive function. Abdominal ultrasound (US) may be a good alternative for the measurement of IAF and SAF compared to the gold standard of CT or Magnetic Resonance Imaging (MRI), due to no radiation exposure, low costs and general availability of US in fertility and antenatal clinics. Validation studies showing that US is a reliable tool for the measurement of IAF and SAF when compared to CT or MRI, were mostly conducted in older populations and have not been performed in women of reproductive age before. **Chapter 4** presents our data on the correlation between the measurements of IAF and SAF by US and CT in women with obesity and infertility at fixed time points (baseline, month 3 and month 6) of a lifestyle programme, as well as the measurements of the changes of IAF and SAF between these time points. The correlation between the IAF measurement by US and CT was good at all time points, but the correlation between the SAF measurement by US and CT was weak. The correlation between the measurement of the changes of IAF by US and CT was only significant between baseline and month 6. The method comparison analyses for the IAF measurements showed good agreement between the US and CT measurements of IAF. In women with obesity and infertility, US can be implemented as a tool for the measurements of IAF, but the measurement of the changes of IAF over time is only accurate if sufficient loss of IAF has occurred. The measurement of SAF by US in its present form is not reliable enough to be used in clinical settings and in future research.

Adipose tissue is not only a site of lipid storage, but a complex endocrine organ expressing and secreting many bioactive peptides, collectively called adipokines. Adipokines, like leptin, adiponectin, interleukin-6 and tumour necrosis factor $\alpha$ may influence female reproduction by an effect on the hypothalamus, the ovary and the endometrium. It can be hypothesized that the measurement of serum adipokine levels could reflect the difference in the body-fat distribution parameters, and the morphology and function of the adipose tissue

compartments. **Chapter 5** elaborates on whether PCOS status (i.e., defined as anovulation and polycystic ovaries on ultrasound and/or hyperandrogenaemia) is a determining factor in the association between body-fat distribution parameters and serum adipokine levels in women with obesity and infertility. Women with anovulatory PCOS had significantly more abdominal obesity (waist circumference and abdominal fat on DEXA scan) and significantly more SAF and no difference in IAF compared to ovulatory non-PCOS controls. In spite of these differences, linear regression analysis of the adipokines with body-fat distribution parameters revealed that PCOS status had no influence on the association between the body-fat distribution parameters and the serum adipokine levels. With higher BMI levels, the measurement of body-fat compartments and serum adipokine levels may not reflect the metabolic and reproductive consequences of obesity due to adipose tissue becoming dysfunctional. Dysfunctional adipose tissue leads to fat redistribution from IAF to SAF and ultimately to accumulation in ectopic fat sites like the liver, pancreas and skeletal muscle. Fat accumulation in ectopic sites is especially associated with IR and an adverse metabolic risk profile. The present study shows more liver-fat accumulation on US in women with anovulatory PCOS compared to the ovulatory non-PCOS controls. Assessment of ectopic fat site deposition, like hepatic fat accumulation, may be a tool to identify women with a further increase in risk of adverse reproductive outcome.



Lifestyle intervention and weight loss in women who are overweight or obese improves the chances of spontaneous conception in anovulatory and ovulatory women. Pre-pregnancy weight loss and lifestyle intervention during pregnancy leads to a decrease in obesity-related pregnancy complications. Weight loss in anovulatory women who are overweight or obese leads to resumption of ovulation in about 60% of women. On average, 5–10% loss of initial body weight is associated with resumption of ovulation which is facilitated by a decrease in IR and hyperandrogenaemia. In women with PCOS, 5% loss of abdominal fat is required for resumption of ovulation, but it is not clear whether differential loss of IAF or SAF contributes to resumption of ovulation. **Chapter 6** outlines our results comparing the changes in body-fat distribution parameters, and especially IAF and SAF, in a group of obese anovulatory women with PCOS who resumed ovulation to those who remained anovulatory during a 6-month lifestyle programme. Resumption of ovulation was associated with 12% and 19% loss of IAF at month 3 and month 6, respectively, whilst the women who remained anovulatory lost 5% and 9% of IAF at month 3 and month 6, respectively. Loss of SAF was not associated with resumption of ovulation. Future studies should assess the combination of diet and structured exercise programs aimed at loss of IAF and improvement of IR for the resumption of ovulation.

Women with infertility who are overweight or obese experience great difficulty to achieve and maintain weight loss. A multifactorial approach based on diet, exercise and behaviour modification is advised to help these women achieve weight loss. Some women however do not achieve sufficient weight loss to reduce the obesity-related pregnancy complications and to reach the target BMI for fertility treatment required by some fertility clinics. The combination of a lifestyle intervention programme with weight loss medication achieves more weight loss than a lifestyle programme alone. Orlistat, the only FDA approved anti-obesity drug should not be used in women who plan to conceive, because its safety during early pregnancy has not been proven. Previous studies have indicated that metformin therapy may contribute to weight loss. In anovulatory women with PCOS, metformin has been studied extensively and has been shown to contribute to resumption of ovulation in a subset of patients. Information on the safety of metformin use in the first trimester of pregnancy is reassuring. In women with obesity and infertility, metformin treatment may therefore be considered as an adjunct to lifestyle intervention in order to achieve more weight loss. In **Chapter 7** we performed a systematic review to assess whether in women of reproductive age who are overweight or obese, treatment with insulin-sensitizing agents contributes to weight loss in comparison to placebo and diet and/or a lifestyle modification programme. This review showed that metformin treatment leads to a significant decrease in BMI when compared to placebo. Treatment with high-dose metformin (>1500 mg/day) revealed a more pronounced decrease in BMI when compared to the low-dose metformin (≤1500 mg/day) studies. Treatment for less than 8 weeks did not show a significant decrease in BMI, even after excluding the trials on low-dose metformin. Metformin treatment compared to diet as a co-intervention did not show a significant decrease in BMI.

In women with obesity and infertility undergoing a lifestyle programme, drop-out is a major limiting factor in achieving the maximal weight loss for the improvement of female reproductive outcome. More studies should be performed to evaluate the patient-related factors that lead to high chances of drop-out and to improve the adherence to lifestyle intervention programs.

In conclusion, obesity is a heterogeneous disorder and the female reproductive consequences of obesity are determined by body-fat distribution and especially accumulation of IAF. With increasing BMI, adipose tissue becomes dysfunctional, leading to redistribution of fat to ectopic fat sites like the liver, skeletal muscles and pancreas. Serum adipokine levels do not reflect body-fat distribution and the dysfunction of the different body-fat compartments. Future studies in women with obesity should measure IAF and liver-fat accumulation by means of US to assess their role in the female reproductive consequences of obesity.

Fertility clinics should offer a structured lifestyle programme to women with obesity and infertility with the aim to achieve sufficient weight loss in order to increase the chance of spontaneous conception and to decrease obesity-related pregnancy complications. In view of the serious obesity-related pregnancy complications, women with a BMI $\geq$35 kg/m$^2$ should not undergo fertility treatment. In women with severe or morbid obesity who have great difficulty achieving sufficient weight loss, weight-loss medication including treatment with metformin may help them to achieve more weight loss, and in a select group of women with a BMI ≥40 kg/m$^2$ bariatric surgery may be considered.

# Samenvatting

De gevolgen van wereldwijd in toenemende mate voorkomend overgewicht (BMI 25–29.9) en obesitas (BMI ≥30) zijn zeer zorgelijk voor de vrouwelijke voortplanting. Deze gevolgen maken het nodig dat net als in andere disciplines ook gynaecologen prioriteit geven aan deze chronische ziekte in klinische behandelingsprotocollen en wetenschappelijk onderzoek. Bij vrouwen met obesitas zijn de kansen op spontane conceptie lager en is er een verhoogd risico op miskramen, in tegenstelling tot vrouwen van normaal lichaamsgewicht. Hierdoor hebben meer vrouwen met obesitas een vruchtbaarheids-behandeling nodig, terwijl deze patiënten tegelijkertijd meer gonadotrofines voor ovariële hyperstimulatie nodig hebben en minder zwangerschappen en levend geboren kinderen verkrijgen met de ingestelde behandelingen. Deze aan obesitas gerelateerde zwanger-schapscomplicaties vormen een grote zorg vanwege de toename van perinatale sterfte en zelfs van moedersterfte in gevallen van ernstige (BMI 35–39.9 kg/m$^2$) en morbide (BMI ≥40 kg/m$^2$) obesitas. Obesitas tijdens zwangerschap leidt bij nakomelingen tot een verhoogde kans op ziektes op de lange termijn vanwege het ongunstige intra-uterien milieu tijdens de foetale ontwikkeling.

De studies in dit proefschrift werden uitgevoerd om de verschillende aspecten van obesitas en vrouwelijke onvruchtbaarheid te onderzoeken. Het belangrijkste aandachtspunt was de rol en de invloed van de verdeling van lichaamsvet, in het bijzonder intra-abdominaal vet (IAF) en subcutane abdominaal vet (SAF), bij vrouwen met obesitas en onvruchtbaarheid die een leefstijlprogramma ondergingen.

Gezien de mogelijke gevolgen van obesitas bij vrouwen die een vruchtbaarheids-behandeling ondergaan, is het aannemelijk dat obesitas een negatieve invloed heeft op de kosteneffectiviteit van vruchtbaarheidsbehandeling. **In Hoofdstuk 2** wordt een model ontwikkeld om de kosten van vruchtbaarheidsbehandelingen (d.w.z. ovulatie-inductie, intra-uteriene inseminatie en in-vitrofertilisatie) en van zwangerschapscomplicaties afzonderlijk te berekenen voor ovulatoire en anovulatoire vrouwen, met respectievelijk normaal gewicht, overgewicht en obesitas. De berekende kosten per levend geboren kind bij vrouwen met obesitas waren vergeleken met vrouwen met een normaal gewicht respectievelijk € 6045 en € 3016 bij anovulatoire vrouwen, en € 10.355 en € 6096 bij ovulatoire vrouwen. Toekomstige prospectieve studies zullen moeten uitwijzen of leefstijlinterventies gericht op gewichtsverlies voorafgaand aan een vruchtbaarheids-behandeling de kosteneffectiviteit van vruchtbaarheidsbehandelingen per levend geboren kind bij vrouwen met obesitas zullen verbeteren.

Obesitas is een heterogene aandoening waarbij accumulatie van vet rond de buik (abdominale obesitas) in verband wordt gebracht met een hoog cardiometabool risicoprofiel en ongunstige uitkomsten van voortplanting bij vrouwen. Abdominale obesitas is gerelateerd aan onvruchtbaarheid en een toename van zwangerschapscomplicaties. Bij abdominale obesitas wordt de accumulatie van IAF, en niet van SAF, in verband gebracht met insulineresistentie (IR) en een hoog cardiometabool risicoprofiel. Er is beperkt bewijs dat bij vrouwen met abdominale obesitas de overmaat aan IAF een nadelig effect heeft op de voortplanting bij vrouwen. In **Hoofdstuk 3** hebben we de invloed bepaald van de vetparameters (in het bijzonder IAF en SAF, gemeten met behulp van een CT-scan van het abdomen) op anovulatie bij vrouwen met obesitas en onvruchtbaarheid. Ondanks vergelijkbare BMI's hadden anovulatoire vrouwen een hogere tailleomtrek, significant meer romp- en abdominaal vet op een DEXA-scan en beduidend meer SAF dan ovulatoire vrouwen. Er was geen significant verschil in het IAF volume tussen de ovulatoire en anovulatoire groepen. Na multipele logistische regressieanalyses konden alleen rompvet en SAF in verband gebracht worden met anovulatie. Dit verband is alleen aangetoond voor de omvang van deze vetcompartimenten en het weerspiegelt niet noodzakelijkerwijs de metabole effecten van deze compartimenten op de voortplanting.

Betrouwbare metingen van IAF en SAF bij vrouwen met obesitas en onvruchtbaarheid zijn essentieel voor een adequate evaluatie van het effect van deze vetcompartimenten op de vrouwelijke vruchtbaarheid. Abdominale echografie kan een goed alternatief zijn voor het meten van IAF en SAF in vergelijking met de gouden standaard van CT of MRI, omdat er geen blootstelling aan straling is, de kosten laag zijn en de techniek in vruchtbaarheids- en prenatale klinieken algemeen beschikbaar is. Validatiestudies waaruit blijkt dat echografie een betrouwbaar instrument is voor het meten van IAF en SAF in vergelijking met CT of MRI zijn meestal uitgevoerd in oudere populaties en deze zijn nog niet eerder uitgevoerd bij vrouwen in de vruchtbare leeftijd. **Hoofdstuk 4** vermeldt onze gegevens over de correlatie tussen de metingen van IAF en SAF met behulp van echografie en CT bij vrouwen met obesitas en onvruchtbaarheid op vaste tijdstippen (start van de evaluatie, maand 3 en maand 6) tijdens een leefstijlprogramma, evenals de metingen van veranderingen in IAF en SAF tussen deze tijdstippen. De correlatie tussen de IAF-metingen met behulp van echografie en CT was sterk op alle tijdstippen, maar de correlatie tussen de SAF-metingen met behulp van echografie en CT was gering. De correlatie tussen de meting van veranderingen in de tijd van IAF met behulp van echografie en CT was alleen significant tussen de start van de evaluatie en maand 6. De Bland-Altman analyse voor de IAF-metingen toonden een goede overeenkomst tussen de echografie- en CT-metingen. Bij vrouwen met obesitas en onvruchtbaarheid kan abdominale echografie geïmplementeerd worden als een instrument voor het meten van IAF, maar de meting van IAF veranderingen over een langere periode van follow-up is alleen nauwkeurig als er voldoende verlies van

IAF heeft plaatsgevonden. De metingen van SAF met behulp van echografie zijn in de huidige vorm niet betrouwbaar genoeg voor gebruik in klinische toepassing en voor wetenschappelijk onderzoek.

Vetweefsel is niet alleen een plaats voor lipide opslag, maar een complex endocrien orgaan dat veel bioactieve peptiden – adipokines genoemd – produceert en afscheidt. Adipokines zoals leptine, adiponectine, interleukine-6 en tumor necrosis factor α kunnen de vrouwelijke vruchtbaarheid beïnvloeden door hun effect op de hypothalamus, de eierstokken en het endometrium. Serum-adipokine-niveau metingen zouden het verschil in verdeling van lichaamsvetparameters en de morfologie en functie van de vetweefsel-compartimenten kunnen weerspiegelen. **Hoofdstuk 5** gaat verder in op de vraag of PCOS-status (gedefinieerd als anovulatie, polycysteuze ovaria op echografie en/of hyperandrogenisme) een bepalende factor is in de relatie tussen de verdeling van lichaamsvetparameters en serum-adipokine-niveaus bij vrouwen met obesitas en onvruchtbaarheid. Anovulatoire PCOS patiënten hadden significant meer abdominale obesitas (tailleomtrek en abdominaal vet op de DEXA-scan) en significant meer SAF maar toonden geen verschil in IAF vergeleken met ovulatoire non-PCOS-controles. Ondanks deze verschillen liet een lineaire regressieanalyse van de adipokines met de lichaamsvetparameters zien dat de PCOS-status geen invloed had op het verband tussen de lichaamsvetverdeling en de adipokine-serum-niveaus. Bij hogere BMI waarden weerspiegelen de metingen van lichaamsvetcompartimenten en serum-adipokine-niveaus mogelijk niet de gevolgen voor cardiometabole risico's en de voortplanting omdat het vetweefsel disfunctioneel wordt. Disfunctioneel vetweefsel leidt tot een herverdeling van vet van IAF naar SAF en uiteindelijk tot accumulatie op ectopische vetlocaties zoals in de lever, pancreas en skeletspieren. Vetaccumulatie op ectopische locaties wordt vooral in verband gebracht met IR en een schadelijk metabool risicoprofiel. Vergeleken met de ovulatoire non-PCOS-controles, laat deze studie meer accumulatie van levervet zien op echo's van vrouwen met anovulatoire PCOS. Meting van vetophoping op ectopische locaties, zoals in de lever, kan een instrument zijn om vast te stellen welke vrouwen een bijkomend risico hebben op de aan obesitas gerelateerde negatieve gevolgen voor de vruchtbaarheid.

Leefstijlinterventie met gewichtsverlies bij vrouwen met overgewicht of obesitas verhoogt de kans op een spontane conceptie bij anovulatoire en ovulatoire vrouwen. Gewichtsverlies voorafgaand aan de zwangerschap en leefstijlinterventie tijdens de zwangerschap leidt tot een daling van de aan obesitas gerelateerde zwangerschapscomplicaties. Gewichtsverlies bij anovulatoire vrouwen met overgewicht of obesitas leidt tot herstel van de ovulatie bij ongeveer 60%. Gemiddeld genomen gaat een verlies van 5–10% van het aanvangsgewicht gepaard met herstel van de ovulatie, gefaciliteerd door een afname in IR en

hyperandrogenisme. Bij vrouwen met PCOS is 5% verlies van abdominaal vet nodig voor herstel van de ovulatie, maar het is niet duidelijk of differentieel verlies van IAF of SAF hieraan bijdraagt. **Hoofdstuk 6** schetst onze resultaten van de vergelijking tussen de veranderingen in de verdeling van lichaamsvetparameters – en in het bijzonder IAF en SAF – bij twee groepen anovulatoire vrouwen met PCOS en obesitas, waarvan de ene groep herstel van de ovulatie kreeg en de andere groep anovulatoir bleef tijdens een leefstijlprogramma van 6 maanden. Herstel van de ovulatie kon in verband gebracht worden met respectievelijk 12% en 19% verlies van IAF in maand 3 en maand 6, terwijl de vrouwen die anovulatoir bleven, respectievelijk 5% en 9% IAF verloren in maand 3 en maand 6. Het verlies van SAF was niet gerelateerd aan herstel van de ovulatie. Toekomstige studies zouden de combinatie van dieet en gestructureerde trainings-programma's gericht op het verlies van IAF en verbetering van IR voor het herstel van ovulatie moeten onderzoeken.

Vrouwen met onvruchtbaarheid en overgewicht of obesitas hebben veel moeite met het realiseren en handhaven van gewichtsverlies. Om deze vrouwen te helpen gewichtsverlies te bewerkstelligen wordt een multifactoriële benadering geadviseerd, gebaseerd op dieet, lichaamsbeweging en gedragsverandering. Sommige vrouwen realiseren echter niet voldoende gewichtsverlies om de kans op zwangerschapscomplicaties te verminderen die te maken hebben met obesitas, en om de gewenste BMI te bereiken die door sommige klinieken vereist wordt voor vruchtbaarheidsbehandeling. Er zijn aanwijzingen dat de combinatie van een leefstijlinterventieprogramma met medicatie gericht op gewichtsverlies in de algemene populatie adipeuze patiënten tot meer gewichtsverlies leidt dan alleen een leefstijlinterventieprogramma. Orlistat, het enige FDA goedgekeurde geneesmiddel om gewichtsverlies te bevorderen, dient niet gebruikt worden door vrouwen die van plan zijn zwanger te worden, omdat de veiligheid van dit geneesmiddel gedurende de vroege zwangerschap niet is aangetoond. Eerdere studies hebben laten zien dat een behandeling met metformine kan bijdragen aan gewichtsverlies. Metformine is uitgebreid onderzocht bij anovulatoire vrouwen met PCOS en er is aangetoond dat metformine bijdraagt tot herstel van de ovulatie bij sommigen van deze patiënten. Gegevens over de veiligheid van het gebruik van metformine in de eerste 3 maanden van de zwangerschap zijn geruststellend. Behandeling met metformine bij vrouwen met obesitas en onvruchtbaarheid kan derhalve beschouwd worden als een aanvulling op leefstijlinterventie om meer gewichtsverlies te bereiken. In **Hoofdstuk 7** hebben we een systematische review uitgevoerd om te bepalen of bij vrouwen in de vruchtbare leeftijd die overgewicht of obesitas hebben een behandeling met medicatie die de insulinegevoeligheid doet toenemen bijdraagt tot meer gewichtsverlies. In de systematische review is de medicatie die de insulinegevoeligheid doet toenemen vergeleken met een placebo en dieet en/of een leefstijl-interventieprogramma. Deze review toonde aan dat een behandeling met metformine leidt

tot een significante afname van de BMI in vergelijking met het gebruik van een placebo. Behandeling met een hoge dosis metformine (>1500 mg/dag) liet een grotere afname van de BMI zien in vergelijking met behandelingen met een lage dosis (≤1500 mg/dag). Een behandeling van minder dan 8 weken liet geen significante daling in de BMI zien, ook niet na uitsluiting van studies met een lage dosis metformine. Een behandeling met metformine vergeleken met een dieet als co-interventie liet geen significante afname van de BMI zien.

Bij vrouwen met obesitas en onvruchtbaarheid die een leefstijlinterventieprogramma ondergaan, is voortijdige uitval een belangrijke beperkende factor bij het niet bereiken van maximaal gewichtsverlies. Er zullen eerst meer studies moeten worden uitgevoerd naar patiënt-gerelateerde factoren die leiden tot grote kans op uitval, om vervolgens het voltooien van leefstijlinterventieprogramma's te kunnen verbeteren.

Samenvattend: obesitas is een heterogene aandoening en de gevolgen ervan voor de vrouwelijke voortplanting worden bepaald door de verdeling van lichaamsvet en in het bijzonder door de accumulatie van IAF. Bij een toename van de BMI wordt vetweefsel disfunctioneel, dit leidt tot een herverdeling van vet naar ectopische vetlocaties zoals de lever, skeletspieren en pancreas. Serum-adipokine-niveaus weerspiegelen niet de verdeling van lichaamsvet en de disfunctie van de verschillende lichaamsvet-compartimenten. Toekomstige studies bij vrouwen met obesitas zouden IAF en de ophoping van levervet kunnen meten met behulp van echografie, om te bepalen welke rol zij spelen bij de gevolgen voor de vrouwelijke voortplanting. Fertiliteitsklinieken zouden een gestructureerd leefstijlinterventieprogramma moeten bieden aan vrouwen met obesitas en onvruchtbaarheid met het doel voldoende gewichtsverlies te bereiken, zodat de kans op spontane zwangerschap wordt verhoogd en de zwangerschapscomplicaties die met obesitas samenhangen, worden verminderd. Gezien de ernstige, aan obesitas gerelateerde zwangerschapscomplicaties zou aan vrouwen met een BMI $\geq$35 kg/m$^2$ geen vruchtbaarheidsbehandeling moeten worden aangeboden. Bij vrouwen met een ernstige of morbide obesitas die grote problemen hebben met het bereiken van voldoende gewichtsverlies kan medicatie gericht op gewichtsverlies, waaronder behandeling met metformine, helpen om meer gewicht te verliezen, en bij een selecte groep vrouwen met een BMI ≥40 kg/m$^2$ kan bariatrische chirurgie worden overwogen.

# Chapter 10

# Acknowledgements and Curriculum vitae

# Acknowledgements

When writing a PhD thesis at the age of 51 years with a career reaching across continents and countries, a long list of acknowledgements is inevitable.

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Een bijzondere wetenswaardigheid is dat dit WOW! onderzoek, waarin vele anovulatoire patiënten met PCO participeerden, gezien kan worden in een tijdslijn die ingezet werd in 1968 toen de eerste anovulatoire patiënten in het UMCG werden behandeld met clomifeencitraat. Dr. R. Lappöhn, gynaecoloog in ruste, heeft zijn proefschrift getiteld "Stoornissen in de follikelrijping bij ovulatie-inductie met Clomipheen" gewijd aan de behandeling van PCO patiënten met clomifeencitraat en wigexcisies. Hierbij maakt hij ook melding van spontane zwangerschappen na gewichtsreductie; "wij allen staan op de schouders van de reuzen die ons zijn voorgegaan"!

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# Curriculum vitae

Walter Kuchenbecker was born on the 24th of February 1962 in Windhoek and matriculated at the Secondary School Swakopmund in Namibia. He studied medicine at the University of Pretoria, South Africa, obtaining the MBChB degree in 1985. This was followed by a year of internship at the Ga-Rankuwa- and Sebokeng hospitals in South Africa. Thereafter he worked as a medical officer at the Okakarara hospital (part of compulsory national military service) and the Windhoek State Hospital in Namibia. During the period of working as a general practitioner in Vanderbijlpark, South Africa, he was accepted to follow the gynaecology training at the University of Pretoria. In 1997 he obtained the FCOG (ZA) degree of the College of Medicine and the MMed (Ob/Gyn) (cum laude) degree of the University of Pretoria, South Africa. After qualifying as a gynaecologist he followed a 2-year advanced training in reproductive medicine at the University of Pretoria. During these 2 years he spent 2 months at the Gasthuisberg Universitaire Ziekenhuis, Leuven in Belgium obtaining further knowledge in reproductive medicine and endoscopic surgery. From 1999 he worked as a gynaecologist at the Vitalab fertility clinic in Johannesburg, South Africa. After emigrating to The Netherlands in January 2001, he worked as a gynaecologist at the Bethesda ziekenhuis in Hoogeveen. From January 2003 up to April 2005 he also worked at the fertility clinic of the University Medical Center Groningen for 1-2 days per week. In May 2005 he joined the Isala Clinics in Zwolle where he works as a gynaecologist and since February 2006 as reproductive medicine specialist.

Walter Kuchenbecker has three children, Gabriele born in1990, Matthias born in1992 and Simone born in1993. Claudia Kemme is his friend and companion.

Walter Kuchenbecker werd op 24 februari 1962 in Windhoek, Namibië, geboren en volgde de middelbare school in Swakopmund in Namibië. Hij studeerde geneeskunde aan de Universiteit van Pretoria, Zuid Afrika, en studeerde af in 1985. Vervolgens werkte hij een jaar in de Ga-Rankuwa- en Sebokeng ziekenhuizen in Zuid Afrika. Hierna werkte hij als basisarts in het Okakarara ziekenhuis (verplichte militaire dienst) en in het Windhoek State Hospital in Namibië. Na een periode waarin hij als huisarts werkzaam was in Vanderbijlpark, Zuid Afrika, kon hij met de opleiding Gynaecologie beginnen aan de Universiteit van Pretoria. In 1997 behaalde hij de FCOG (ZA) graad bij het College of Medicine en de MMed (Ob/Gyn) graad (cum laude) aan de Universiteit van Pretoria. Hierna volgde hij de aanvullende opleiding in de voortplantingsgeneeskunde van 2 jaar aan

de Universiteit van Pretoria. Tijdens deze 2 jaren verbleef hij 2 maanden in het Gasthuisberg Universitaire Ziekenhuis in Leuven in België om zich verder te bekwamen in de voortplantingsgeneeskunde en de endoscopische chirurgie. Vanaf 1999 werkte hij als gynaecoloog in de Vitalab fertiliteitskliniek in Johannesburg, Zuid Afrika. Na de emigratie naar Nederland in januari 2001 werkte hij als gynaecoloog in het Bethesda ziekenhuis in Hoogeveen. Van januari 2003 tot april 2005 werkte hij ook gedurende 1–2 dagen per week in de fertiliteitskliniek in het Universitair Medisch Centrum Groningen. Sinds mei 2005 is hij werkzaam als gynaecoloog in de Isala klinieken in Zwolle en in februari 2006 kreeg hij zijn registratie voor het subspecialisme voortplantingsgeneeskunde.

Walter Kuchenbecker heeft 3 kinderen, Gabriele geboren in 1990, Matthias geboren in 1992 en Simone geboren in 1993. Claudia Kemme is zijn vriendin en levensgezellin.